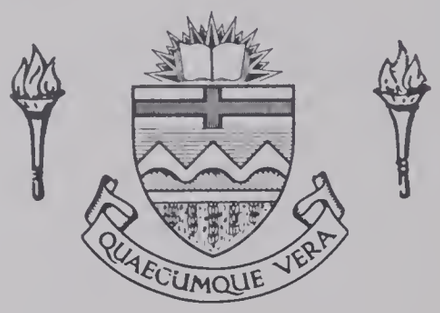

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by

BARRY ALLAN MUNSON

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled THE BIOLOGY OF GOLDEYE, *HIODON ALOSOIDES*, IN THE NORTH SASKATCHEWAN RIVER WITH SPECIAL REFERENCE TO MERCURY CONTAMINATION IN THIS SPECIES OF FISH

submitted by Barry Munson

in partial fulfilment of the requirements for the degree of Master of Science in Pharmacology and Zoology.

To Julie - FOREVER 24/5/69

- B.

ABSTRACT

Goldeye, *Hiodon alosoides*, sampled in 1973 from the North Saskatchewan River in Alberta, contained mercury levels far in excess of the current government limit of 0.5 ppm and significantly higher than other species of fish examined for the same, relatively mercury free environment.

Two possible hypotheses to explain these data are available; either goldeye accumulate mercury in some other environment and migrate into Alberta, or goldeye possess some unusual physiological features which permit a resident population of fish to accumulate and retain mercury from an environment which only has a low level of contamination.

In order to test the first hypothesis, goldeye were caught by line or gill net from the North Saskatchewan River at a variety of different sites from Edmonton to Tobin Lake, Saskatchewan. Some fish were measured, weighed, the age determined and mercury levels in various tissues determined by flameless atomic absorption spectrometry, while, others were tagged and returned to the river. Other species of fish were also examined. The results of this study may be summarized as follows:

(a) Mercury levels in goldeye of similar age and weight did not differ significantly between Tobin Lake and Edmonton, while levels in other species were higher in Saskatchewan.

(b) A decrease in mercury levels of all species in Saskatchewan were observed over the three years of the study, attributable to reduced contamination of Tobin Lake. In Edmonton a similar decrease was observed in goldeye but not in other species.

(c) In Edmonton in the spring most goldeye caught were in the 3-4 year age range. Many females contained eggs or appeared to have recently spawned. Later in the year in Edmonton the numbers of goldeye captured was much reduced. No young-of-the-year were caught.

(d) In Tobin Lake all ages were encountered but in the spring relatively few 3-4 year old fish were caught. Young-of-the-year were common later in the year.

(e) Mercury levels in fish caught in Edmonton were highest in tissues which "fix" mercury (lens, white muscle), while fish in Tobin Lake showed relatively greater amounts in tissues from which mercury is lost more rapidly (e.g., gonad).

These data are consistent with the concept that the high levels of mercury in Alberta goldeye arise from contamination of the fish in Saskatchewan followed by westward migration. It is suggested that this migration takes place in the spring, that spawning occurs in Alberta and the single buoyant eggs of this species are carried downstream and hatching and maturation of the fish occur in Saskatchewan. After spawning in Alberta a further easterly migration to overwintering spots could take place. Tag returns are consistent with this hypothesis, but further work is needed to establish this suggestion.

The contribution of goldeye physiology to the contamination of these species by mercury was determined by acute exposure of captured goldeye to Hg^{203} and subsequent determination of urinary excretion of Hg^{203} via an indwelling catheter and, later, sacrifice of the fish and determination of tissue distribution and quantity of Hg^{203}. Similar experiments were conducted on rainbow trout. While these experiments do not parallel well the exposure of the natural population to mercury, the results obtained from goldeye and rainbow trout were not significantly different, and there is thus no reason at this time to believe that goldeye accumulate and retain mercury to a greater extent than other species. It would seem most probable that the original observations which gave rise to this study are best explained by contamination of the fish by a well-established source of environmental mercury in Saskatchewan and subsequent migration to Alberta.

"In this supercharged world of high inventiveness and continual demands "for more", it is perhaps too much to expect that administrative control will ever progress much beyond a hesitating movement from crisis to crisis; but it is too bad when research, the only really stablizing force that administration can call upon, permits itself to be dominated by a spirit of breathless urgency. Research, pure or applied, must, of course, respond to felt needs, but only with one part of its complement. For the rest it must continue devotion to its centuries-old concept of seeking principles rather than instances, understanding rather than mere factual knowledge. The charge usually referred to as *Occam's razor-entia non sunt multiplicanda praeter neccessitatem* - is still its ideal."

Lee 1973

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Environmental contamination encompasses such a wide diversity of specializations that no one individual can possibly be expected to possess the complete background necessary to successfully conduct meaningful and useful research in this field. It is for this reason that this project was initiated as an interdisciplinary study.

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Comradeship technical assistance and a continuous source of ideas were contributed to this project by the summer assistants, who braved the wilds of Saskatchewan, my dexterous handling of an open boat for a measly pittance. The names on the headstones read; Don Rubuliak, Ron Tanasichuk, Mark Ehrman, John Tuttle and Maury Tymchuk.

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Kath Cook - may your chopper forever hum and that white moose be forever satiated.

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CHAPTER I

GENERAL INTRODUCTION AND HISTORY OF ENVIRONMENTAL MERCURY CONTAMINATION

INTRODUCTION

Headlines

The influence of mercury on human health has a long and involved history.....

Date: Middle 1800's

Location: Manchester, England

- the appearance of an inordinate number of employees of long-standing service showing tremor and mental disease.

Date: Early 1900's

Location: Almadén, Spain

Almadén Mercury Mine

- definitive evidence that longterm exposure in the mine has produced an unusual set of symptoms present in a surprisingly high number of miners - these symptoms involve impairment of locomotor, audio and visual functions.

Date: Early 1950's

Location: Minamata Bay, Japan

- an increasing incidence of congenital deformities coupled with infant mortalities added to the appearance of a substantial number of adults presenting with progressive neurological disorders including locomotor impairment coupled with audio and visual disorders.

10 years later - with deaths from this unknown affliction now approaching thirty - the Japanese government instigates immediate indepth investigations in an attempt to delineate the causative agent or agents and eradicate them.

Date: Early 1960's

Location: Stockholm, Sweden

- officials, fully aware of the Japanese problem, instituted complete research facilities to investigate possible environmental contamination in the light of recent industrial expansion. Presently the only signs evident that the potential for a catastrophe is lurking are the drastic decline in fish-eating bird populations due to sterility and abnormally high mortality rates.

- 1966 - Prominent Swedish scientist Lars Eric Friberg warns North American scientists, "if we would but seek out mercury contamination in air, food and water, we would most assuredly find the problem lurking there, awaiting discovery".

Date: 1969

Location: Alamogordo, New Mexico, U.S.A.

- three children, of a poor New Mexican hog farmer, are admitted to hospital with symptoms which the Japanese have traced to chronic mercury toxicity and termed Minamata Disease.

Subsequent investigations revealed the toxin to be a mercurial compound. The mercury was transmitted to the children through their diet which consisted primarily of pork - pork from hogs fed grain, which had been treated with a mercurial fungicide.

Date: 1970

Location: - Lake St. Claire, Canada

- Wabagoon River System, Canada

- Ottawa River System, Canada

- Lake Winnipeg System, Canada

- Saskatchewan River System, Canada

Governments, private industry, and research institutions commence environmental surveillance and monitoring; the results indicate the necessity for wide-ranging waterway closures to commercial and sport fishery due to alarmingly high levels of mercury contamination.

PHYSICAL PROPERTIES AND ENVIRONMENTAL MERCURY CYCLES

North American society appears to function on a crisis motivated basis and public awareness of the potentially devastating effects of environmental contamination has increased. It is therefore, not surprising that government and research institutions have dramatically increased both their concern and effort to identify the extent of environmental contamination. But, more importantly, they are pursuing programs aimed at determining the mechanism by which contaminants are distributed in and move through the environment to exert their effects on man (Klein, 1972; Bruty et al., 1973).

Mercury, a naturally occurring element in the earth's crust, exists in a dynamic cycle through the air-water-land media which determines the background levels of mercury observed (figure 1). Figure 2 illustrates the effects of overloading one or more of the compartments, thereby altering the equilibrium of the exchange reactions and thus permitting the unequal accumulation of specific forms of mercury in one or more of the compartments (Klein & Goldberg, 1970; Goldberg, 1970; Weiss et al., 1971; Klein, 1972; Peakill & Lovett, 1972; Bruty et al., 1973).

In the interest of a more comprehensive understanding of the complexity of the interaction depicted in Figure 2, that is the movement, distribution and toxicity exhibited by mercurial compounds, a brief review of the physical and chemical properties of mercury and its compounds is presented.

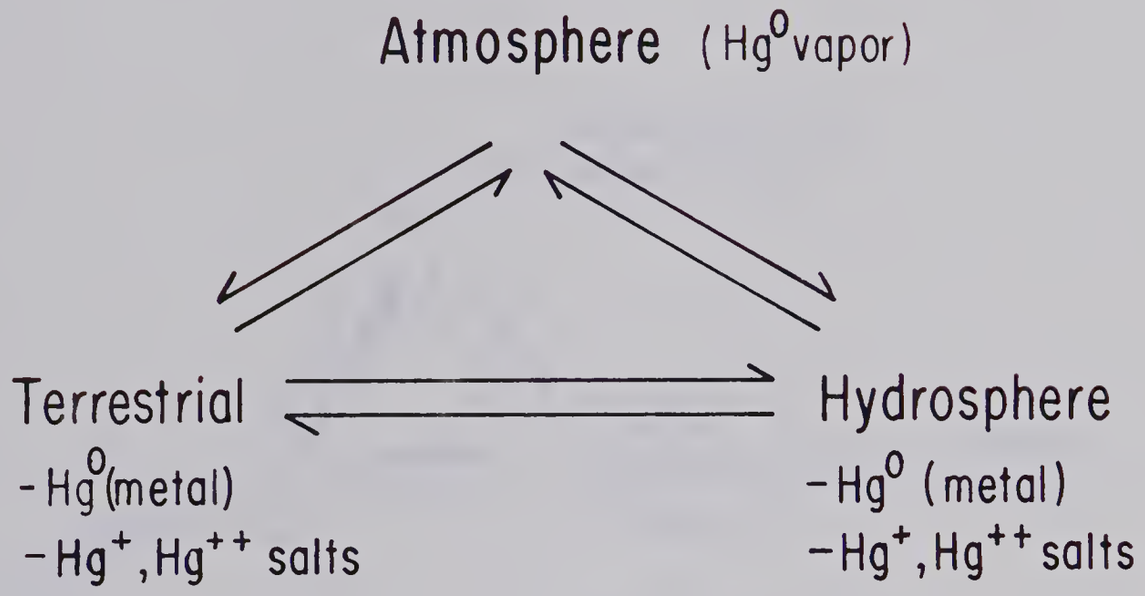

Figure 1 Dynamic Cycle of Mercury through the Air-Water-Land Media Which Determines Background Levels of Mercury

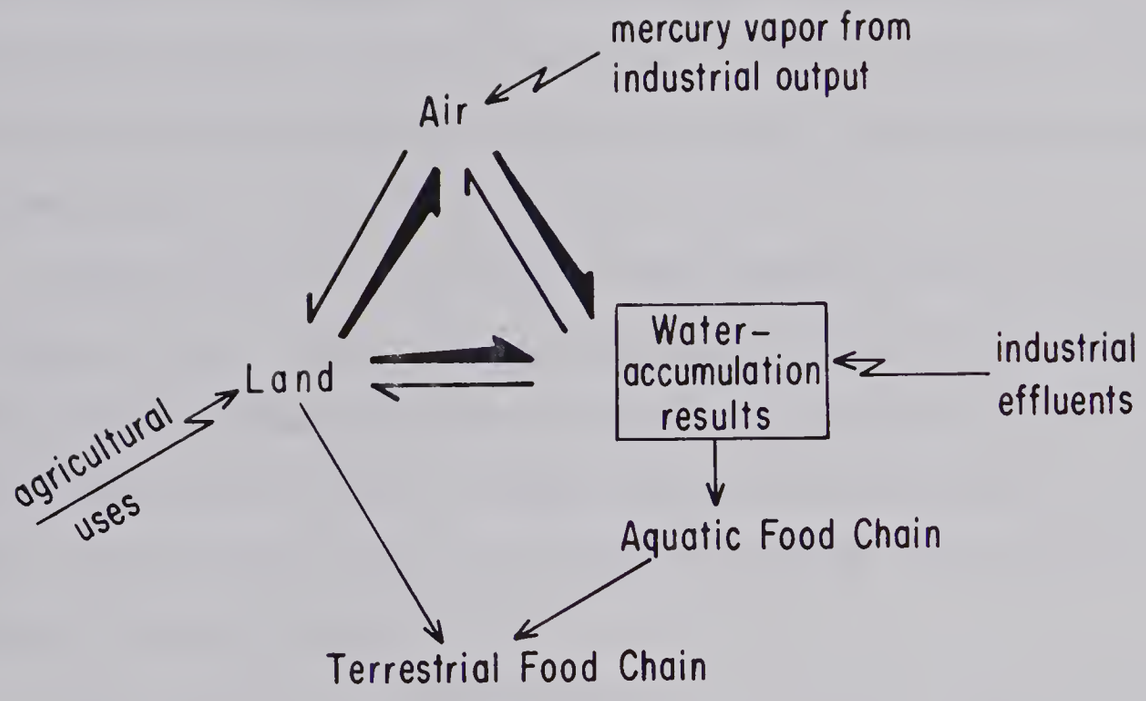

Figure 2 Movement of Mercury Through the Three Environmental Compartments: Illustrating the Effect Upon the Exchange Reactions Following Uncontrolled Input by Man.

Physical and Chemical Properties

Mercury is a Group II-B element with an oxidation state of not higher than 2. It is therefore divalent and usually considered to be a transition element. Because of a low melting point, the metal exists as a liquid at room temperature. This feature, coupled with a high vapour pressure of elemental mercury, imparts a significant volatility rate to the metal. Mercury is readily oxidized to mercurous (Hg^{+}) and mercuric (Hg^{++}) forms which complex with halogens and other elements. These complexes are relatively insoluble in aqueous solutions. The term inorganic mercury is used as a collective term to define the metal itself and the inorganic complexes that it forms (Vallee & Ulmer, 1972).

Organic mercury is defined as mercury covalently bound to aromatic or aliphatic groups. Of all the existing organic mercurials, the simplest, methyl mercury, has proven to be the most toxic (Goodman & Gilman, 1975). This arises from its ease of movement through the environment, the food chain, and the body owing to high solubility in both lipids and water (Vallee & Ulmer, 1972).

Mercury possesses a very high affinity for sulfur, exemplified by the form in which it is mined, cinnabar (84% Hg and 16% S). This affinity for sulfur is primarily responsible for the toxicological properties of this element. The cellular homeostasis is biochemically governed through enzyme-mediated chemical reactions. Sulfur, in the form of sulfhydryl groups (-SH) and disulfide bridges (-S-S-) is a primary constituent of several amino acids. These sulfur-containing amino acids are incorporated into the protein structure of various enzyme systems and following exposure to mercury, the resulting formation of mercury-sulfur bonds inactivates the enzyme. The specific enzymes involved in mercury toxicity have, however, not as yet been conclusively identified (Goodman & Gilman, 1975).

Toxicity Uses - Environmental

In terms of toxicity, mercurial compounds are divided into two basic categories, organic and inorganic. Table 1 lists the properties and toxicities in man of these two classes of mercurial compounds.

Mercury has been subjected to an ever-increasing industrial demand as new and diverse uses for this element have developed in the preceeding five decades. The major sources of this demand are shown in Table 2.

Inevitably, mercury has been introduced into all three environmental compartments with increasing regularity. However it appears that the ramifications of the introduction of mercury into the atmospheric and terrestrial segments have been of minor consequence primarily because both of these systems lack a means of concentrating, transporting and mobilizing mercury. On the other hand, mercury introduced into aquatic ecosystems is presented with ideal modes for dispersion into most trophic levels (Blight, 1970). This, coupled with the fact that surface waters provide a convenient, cheap waste disposal system has provided the basis for extent of mercury contamination observed.

Inorganic mercury usually enters an aquatic environment as mercury metal (Hg°), mercurous (Hg^{+}), or mercuric (Hg^{++}) salts. All of these compounds are heavy and relatively insoluble. It was not unreasonable, therefore, to conclude that upon introduction to an aquatic environment, these compounds would then simply settle into the bottm sediments and be buried forever. Wood *et al.* (1968) revealed that there existed bacteria present in bottom sediments which possessed the ability, by a simply methylation step, to convert inorgainic mercury to methylmercury. Now any future consideration of the introuduction of inorgainc mercury must also include the possible effects related to the distribution of methylmercury. Methylmercury, as noted previously, possesses an enhanced mobility in an aquatic ecosystem which facilitates its incorporation into the food chain mechanism.

TABLE 1. PROPERTIES, TOXICITY AND TREATMENT IN MAN OF INORGANIC AND ORGANIC MERCURIAL COMPOUNDS

	Inorganic	Organic
Absorption from gut	< 10% of total input	> 80% of input
Water solubility	insoluble	highly soluble
Lipid solubility	low	high
Speed of absorption from gut (function of above)	very slowly	45 x as fast
Blood	plasma bound	bound to red blood cells
Excretion (via kidney)	relatively quick	very slowly
½ Life	≃ 30 da.	≃ 60-90 da.
Placenta	crosses poorly	crosses readily
Blood-brain barrier	crosses poorly	crosses readily
Symptoms in Man		
Acute	- grey mouth + oral pain	similar symptoms but even more rarely encountered
Very seldom encountered from industrial contamination	- gastric irritation resulting in emesis (another reason for small incidence)	
	- diarrhea - bloody	
	- haematuria	
	- anuria	
	- severe renal damage ↓ death	

.....cont'd.

Table 1. - cont'd.

	Inorganic	Organic
Treatment:	BAL*+ lavage with sodium formaldehyde sulfoxylate	
Chronic	- stomach pain - general GIT disturbances - loss of appetite - renal damage - neuritis - tremors - anaemia	similar symptoms but with more pronounced neural involvement - including impaired auditory-visual and motor coordination.
Treatment:	BAL N-acetylpenicillamine	later literature suggests a combination of both.
	Some drugs may protect against further renal and neural damage: - selenium - spironolactone	
Prognosis:	- renal damage and neural damage, if caught early, can be reversed to some extent.	neural damage virtually irreversible.

Goodman & Gilman, 1975

---

* BAL - British Anti-lewisite or Dimercaprol

TABLE 2. INDUSTRIAL USES OF MERCURY (ranked in order of importance - as a contamination source)

Chlor-alkali industry - mercury cathode cell continuous flow system to produce high grade, pure caustic soda (NaOH) and chlorine.

catalyst - used to prepare various catalytic salts (oxides, chlorides, sulfates, acetates) which are utilized in the production of poly vinyl chloride urethanes.

This use of Hg is presently the greatest single consumer and pollutor of Hg.

Pulp and paper industry - as a fungicide and slimicide.
- use has been curtailed by government regulation.

Agricultural - seed treatment

Electrical industry - mercury batteries
- alkaline energy cells
- rectifiers, tubes, lamps, switches
- relays, pump seals, valves

Dentistry - restorations - amalgams

Laboratory - general use - thermometers
- manometers

Pharmaceuticals, cosmetics and soaps

Fixatives - for tissues prior to preferential staining.

Figure 3, traces, diagramatically, the distribution of mercury through the environment and into the food chain as a result of bacteria-mediated methylation and direct environmental input.

The concept of food chain magnification, in simple terms, states that the absolute quantities of mercury are distributed over fewer individuals as one ascends the food chain. This results in higher levels of mercury per individual at each successive trophic level of the food chain (Jernelov, 1969; Hartung & Dinman, 1972).

HISTORY

With the delineation of the mercury problem in Japan (Irukayama, 1966), many of the industrial nations finally initiated comprehensive environmental monitoring for mercury contamination. In the late 1960's and early 1970's Sweden, the United States and Canada, all reported widespread environmental mercury contamination (N.S.F. Summer Environmental Study, 1970).

In Canada, Fimreite (1971), then a graduate student at Western University, reported levels of mercury in fish from the Great Lakes and Lake St. Claire to be well in excess of the 0.5 ppm government standard. Suddenly mercury contamination was very much in the headlines with high levels reported from the English, Wabagoon and Ottawa River systems in Ontario (Bligh, 1970). The high levels of mercury found in the Lake Winnipeg system in Manitoba and in the Saskatchewan River system in Saskatchewan also contributed to the apparent mercury panic.

In all of these cases the contamination was traced to one of two sources, either pulp and paper mills which used mercurial fungicides and slimicides, to control mold and fungus growth in the sewage systems or to chlor-alkali industries which used mercury in electrolytic cells.

The mercury crisis spread to Alberta in 1970 when Fimreite (1971) reported very high mercury levels (in excess of 1 ppm) in the livers and breast muscle of upland game birds. The Government of Alberta closed the upland game bird

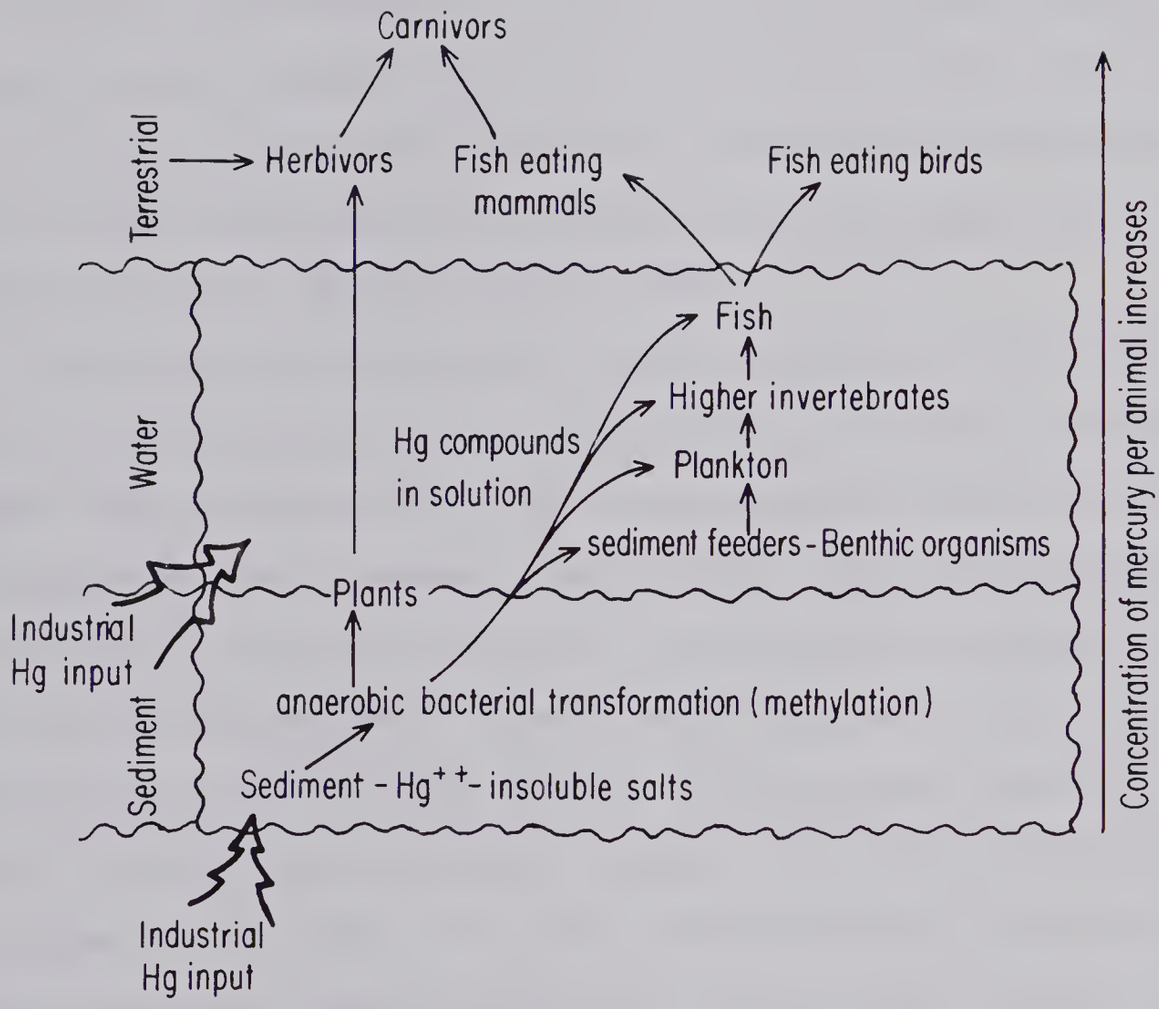

Figure 3 Distribution of Mercury Through the Environment to its Incorporation into the Food Chain

hunting season for one full year. This step was taken in order to determine the source of the contamination and to provide time for the problem to cure itself. The mercury in these birds was traced to their consumption of panogen-treated seed grains. Panogen is a very potent fungicide which contains methyl mercury as its active ingredient (Bligh, 1970).

During the period 1968-1971, a survey of mercury levels in several species of fish in Alberta revealed that several species from specific areas contained levels of mercury significantly above background. Fish collected from the North Saskatchewan River contained by far the highest levels of mercury in excessive numbers (Paterson, 1970).

These observations instigated a more indepth study of the problem. In 1972, a research group under the direction of Dr. E.E. Daniel, commenced an indepth study into the extent of mercury contamination in the North Saskatchewan River in Alberta (Munson & Daniel, 1973). Their results indicated very low levels of mercury were present in or were being introduced into the North Saskatchewan River. However, there definitely existed substantial contamination of the fish fauna found in the North Saskatchewan River. Of all the fish examined, one species - goldeye, *Hiodon alosoides*, possessed the highest mercury levels. If indeed there existed very low levels of mercury in the river water and industrial effluent, where then was the mercury in the fish originating from? Two basic possibilities were proposed in an attempt to explain the high mercury levels found in goldeye.

Firstly, goldeye may accumulate mercury from high environmental levels present outside of Alberta and subsequently migrate into Alberta. Secondly, there may exist some peculiar aspect of goldeye physiology which permits accumulation from the very low environmental levels found in the North Saskatchewan River.

This thesis is an attempt to provide answers to these questions. The problem will be dealt with in four subsequent chapters, each concentrating on a specific aspect of the problem.

Chapter II — Biology of Goldeye

Chapter III — Mercury Levels in Fish from the Saskatchewan River System.

Chapter IV — Physiological and Pharmacological Distribution of Mercury in Goldeye.

Chapter V — General Discussion and Conclusion.

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CHAPTER II

THE BIOLOGY OF GOLDEYE

INTRODUCTION

Goldeye, *Hiodon alosoides*, is a fresh-water fish possessing a deep, laterally compressed body. The surface of the body is covered with large, silvery, cycloid scales which are deciduous (figure 1). Coloration in live specimens ranges from a dark blue-green along the dorsal surface, to a faint gold sheen along the lateral portions of the body (Scott & Crossman, 1973). Goldeye are relatively small fish, maximum length 40 cm and maximum weight 1000 gms, possessing very large anteriorly set eyes, which are a rich gold in color, hence the name.

Goldeye belong to the mooneye family, *Hiodontidae*, whose present distribution is restricted to North America (figure 2). There is only one genus, *Hiodon* and three species, *H. tergisus* northern mooneye; *H. selenops*, southern mooneye; and *H. alosoides*, goldeye; comprising the members of this family (Kennedy & Sprules, 1967).

Historically, goldeye was initially described as a separate species by Rafinesque in 1800 as *Amphiodon alosoides*. Goldeye was described in Canada, initially as *Hydon crysopsis* by Richardson (1836). Subsequent names have included *Hyodon alosoides*, Jordan & Gilbert (1883), a return to the original *Amphiodon alosoides* in 1910 by Jordan & Thompson. Bailey (1956) recommended the present accepted scientific name of *Hiodon alosoides* (Rafinesque), (Kennedy & Sprules, 1967).

"Winnipeg goldeye" or smoked goldeye has been very highly regarded as a connoisseur's delight and it is in this form that the goldeye has found its commercial value. A healthy demand, coupled with a limited supply, has kept the monetary return to the fisherman relatively high, thereby maintaining the goldeye fishery as an economically viable enterprise.

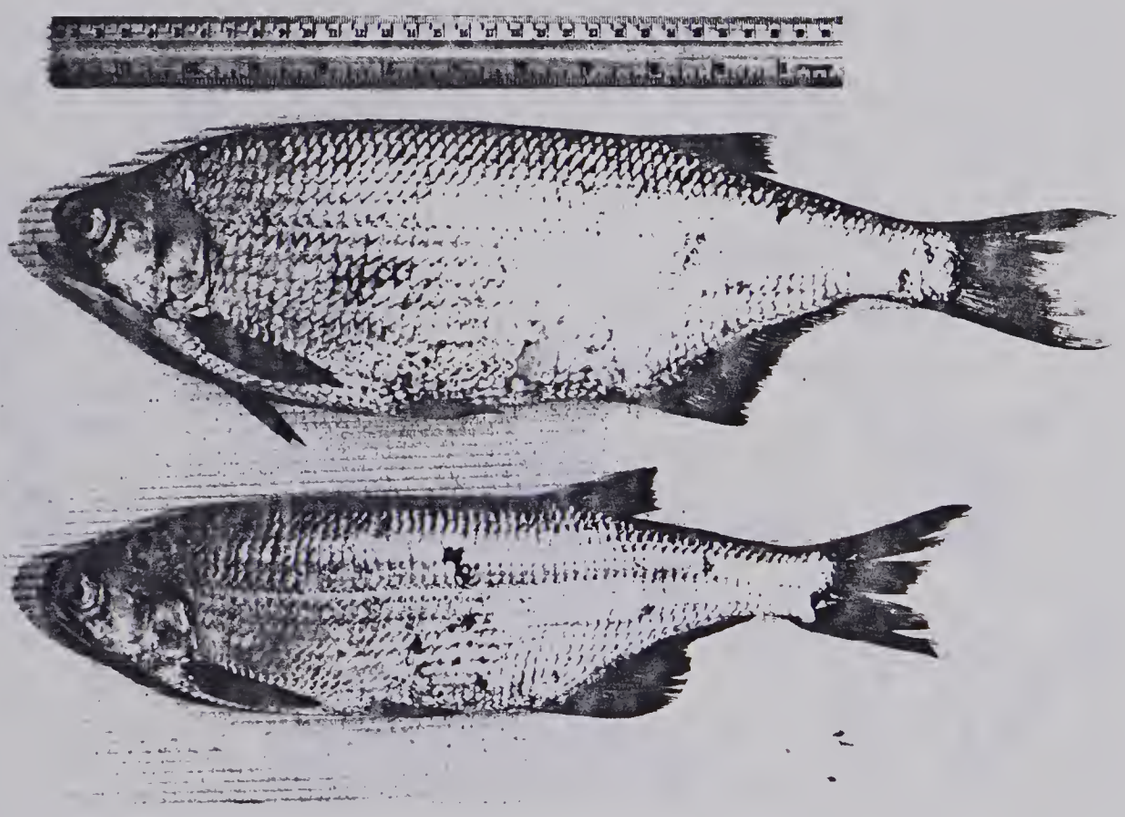

Figure 1. Goldeye, *Hiodon alosoides*

Upper - female

Lower - male

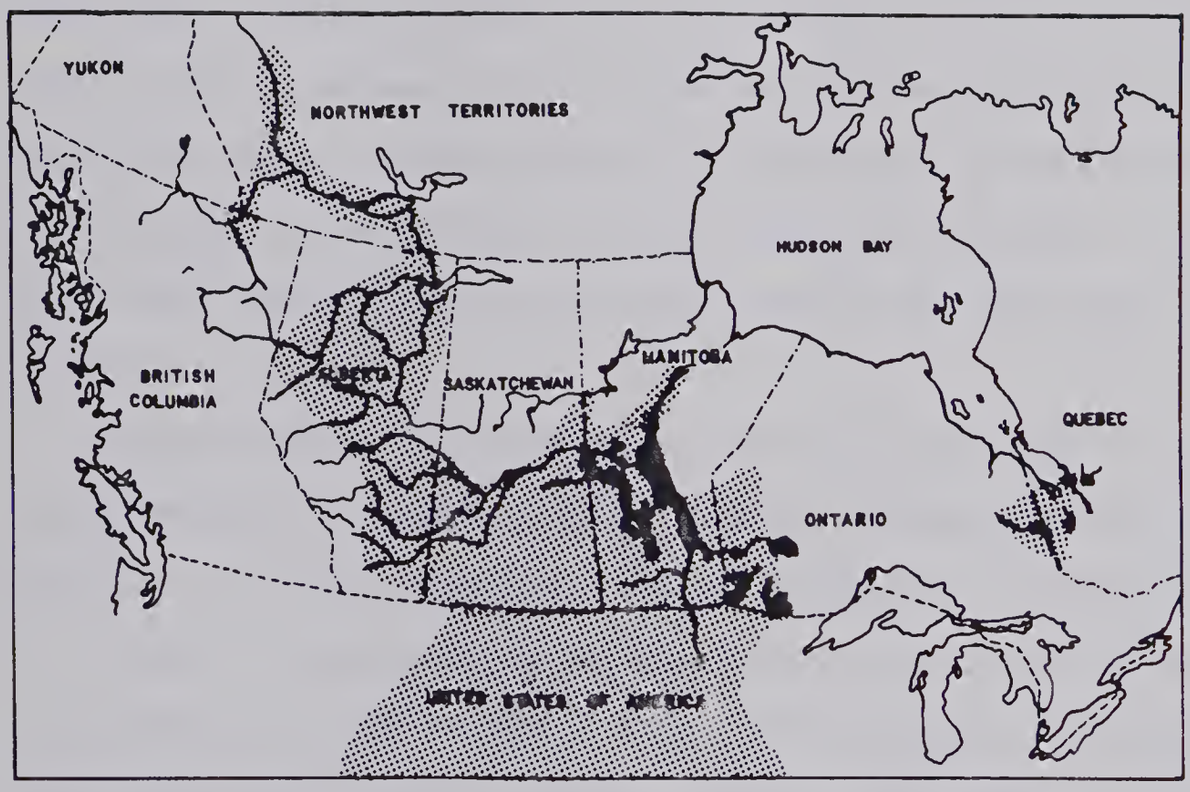

Figure 2 Goldeye, *Hiodon alosoides*

Distribution in North America

(Kennedy & Sprules, 1967)

Information on the general biology of goldeye is restricted to one major publication Kennedy & Sprules (1967) which was an update of the 1947 data by Battle & Sprules (1960). The following data have been compiled from Kennedy & Sprules (1967) as well as several other publications, Kooyman (1972), Donald (1972), Paterson (1966), Paetz & Nelson (1970) and Scott & Crossman (1973).

Goldeye inhabit large rivers, small shallow lakes and the edges of large lakes, frequenting areas which are the most turbid. They have been reported to be most active at dusk and dawn, at which time feeding presumably occurs. Goldeye appear to be omnivorous, as their diet consists of that which is most abundant in their immediate environment. Their preference, however, appears to be insect larvae and other aquatic invertebrates.

Goldeye exhibit sexual dimorphism, as the anterior rays of the anal fin of the male are elongated producing a convex anterior margin to the anal fin. In females the anterior margin is concave or straight (figure 1). Goldeye achieve sexual maturity at 3-4 years and it appears that males may become mature before females. Hinks (1948) suggested that goldeye spawn every second year; however, Kennedy & Sprules (1967) appear convinced that, in the majority of cases, spawning occurs annually. In most areas investigated, especially if averaged on a seasonal basis, the male and female ratio was 1:1. Kennedy & Sprules (1967) reported that the males possessed 1-2 drops of sperm while the female contained upwards of 20,000 eggs. One very interesting feature of the eggs is that they are semi-buoyant, expelled singly and appear to be free-floating (Battle & Sprules, 1960).

It has been suggested by several authors (Kooyman, 1972; Donald, 1972; Fernet, 1973 & pers comm; Kraft, pers comm) that goldeye may migrate considerable distances. This migration appears to be related to the annual spawning cycle.

A literature search concerning the biology of goldeye in the North Saskatchewan River in Alberta is restricted to one small note (Paterson, 1966) and one must therefore apply data collected elsewhere when attempting to determine the status of goldeye in the North Saskatchewan River.

As noted in Chapter 1, goldeye from the North Saskatchewan River exhibited the highest levels of mercury found in fish species in Alberta. These fish may be exposed to mercury elsewhere and subsequently migrate into Alberta (Munson & Daniel, 1973). Two pre-requisites are required for this hypothesis to be valid. First goldeye must routinely migrate large distances and second, there must exist a mercury contaminated area which is within migratable distances of Alberta.

Because of the apparent void in data pertaining to the status of goldeye in the North Saskatchewan River an adequateanswer to the former hypothesis was essentially a matter of speculation. This chapter will therefore attempt to contribute to the general biology of goldeye in the North Saskatchewan River.

MATERIALS AND METHODS

Goldeye were collected by minnow trap, bag seine and gill net at several sites in Alberta and Saskatchewan. These sampling sites included several (6) upstream of Edmonton, Alberta on the North Saskatchewan River (figure 3), and the following sites in Saskatchewan on the North Saskatchewan and Saskatchewan Rivers; Nipawin, Tobin Lake, Maidstone Ferry, Maymont Ferry and the River Forks (figure 4). The gill net stretched mesh size consisted of 0.7, 0.8, 1.0 and 1.4 centimeters. These nets were set parallel to the current of the river and were suspended, by means of floats, to a depth of one foot below the water surface. Depths of the nets ranged from 1.8 to 2.4 meters. These nets were checked and cleared every 30 minutes and remained on site 24 hr/day, 7 days/week for two week periods.

A tag and release program was initiated utilizing a dart tag. A labelled FLOY dart tag was inserted into the dorsal axial musculature posterior to the dorsal fin by a Dennison tagging gun (Floy Tag and Manufacture Co., Washington, U.S.A.).

Fish to be tagged were gill netted and released within 30 minutes of capture. Each fish which received a tag also had the standard length, weight and sex recorded.

In order to determine the effects of the tagging and handling procedure, a compound measuring 200 m x 15 m was constructed in the North Saskatchewan River, utilizing a natural lagoon (figure 5). A 9.0 cm stretched mesh gill net, 25 m long, was also included in the compound to determine whether the goldeye would avoid a gill net after an initial experience with one.

Age of goldeye was determined by projecting a slide, containing 5 scales from each fish, onto a screen and counting the annuli. Precision

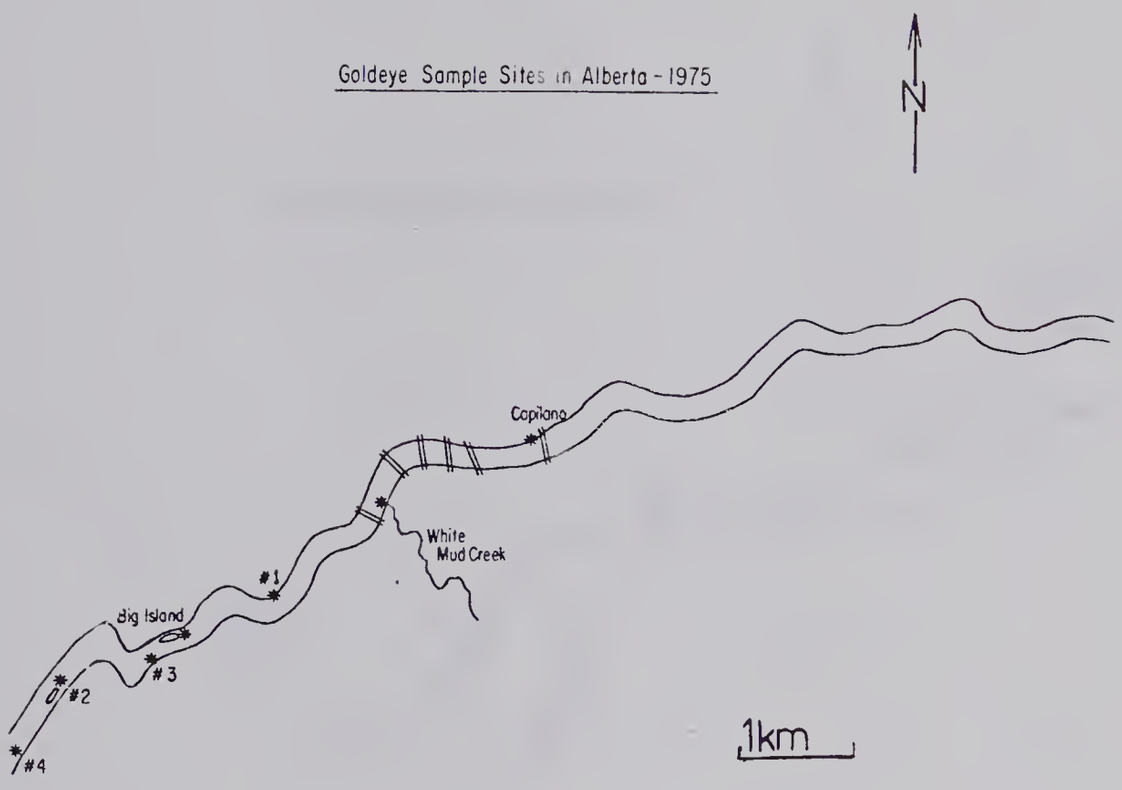

Figure 3 Goldeye Sampling Sites in Alberta (Edmonton Area)

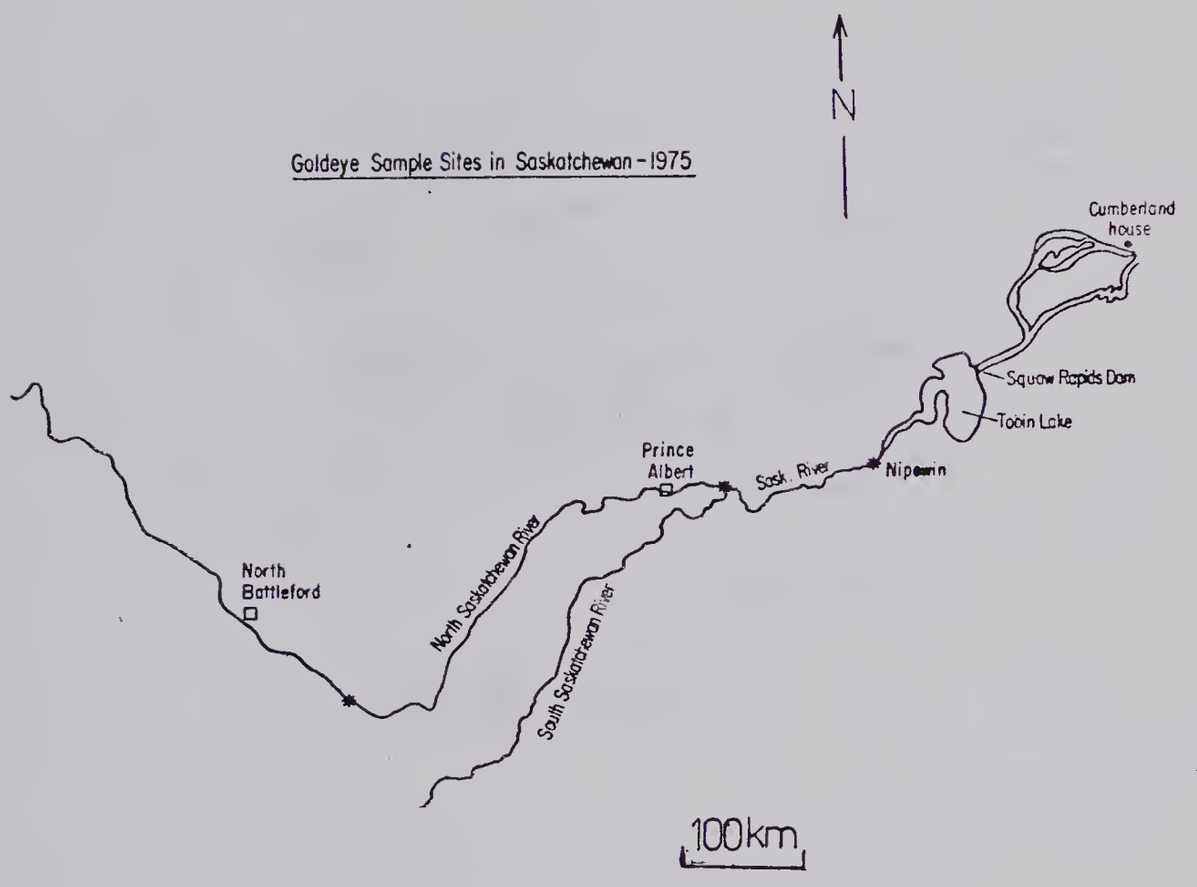

Figure 4 Goldeye Sampling Sites in Saskatchewan

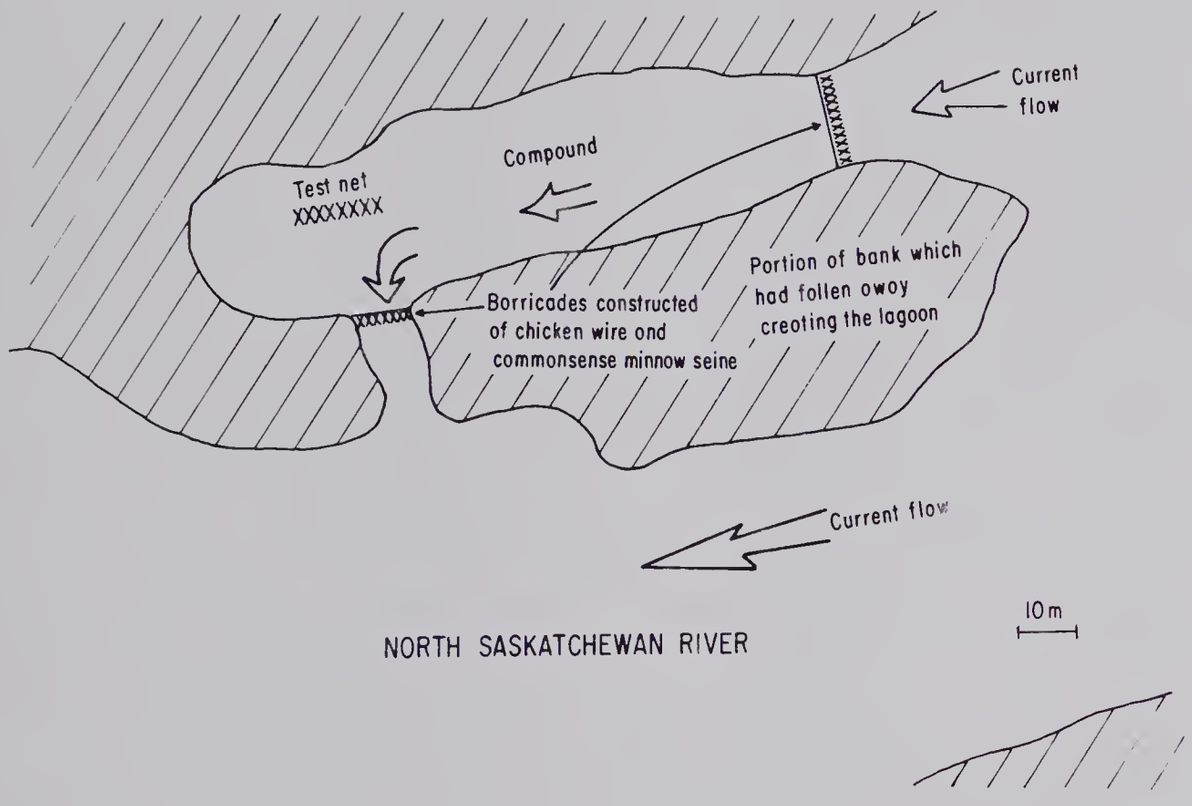

Figure 5 Holding Compound constructed on the North Saskatchewan River near Edmonton, Alberta

of this technique was determined by a double blind procedure* and for goldeye in excess of four years of age was determined to be ± 1 year. For goldeye younger than four years precision was virtually 100%.

* Fifty, previously aged scale samples, were rechecked by the author and at least one other person. The slides containing the scales were marked with only an identification number.

RESULTS

Table 1 summarizes the total catch record of goldeye for the period 1974-1976.

Tagging was conducted during the summers of 1974 and 1975 with a total of 1885 goldeye being tagged and released. In 1974, of the 976 goldeye which were tagged in both provinces, only 6 tags were returned and all of these were from goldeye which had been tagged at Nipawin, Saskatchewan. Of the 6 returns, 2 moved 10-25 km upstream and 4 were recaptured at Squaw Rapids Dam, 175 km downstream. The two fish which moved upstream were in the 2-3 yr old class and were recaptured in July. All the fish which moved downstream were tagged in late June and were all in excess of 4 yrs of age.

In order to determine whether factor(s) other than distance (3000 km) and dilution, were contributing to the low tag return, the test compound (figure 5) was utilized.

Table 2 illustrates that in excess of 50% (15 of 26) of the gill netted and tagged goldeye died within 9 days after capture and handling, whereas < 10% (3 of 36) of the goldeye which were line caught died after the same test period. The 15 goldeye, which died following gill netting, were infected with a fungus which appeared to affect areas of the body damaged by the gill net. Fish alive at the end of the test period were fungus free. The goldeye which expired following line capture did not show any symptoms of fungus infestation.

The ability of goldeye to learn after a single exposure to a gill net was also tested in the compound. The compound was stocked with gill netted goldeye (24 hrs to 3 days previously), who were then exposed to the test net which had been placed in the compound. Within 24 hrs in excess of 90% of these goldeye were recaptured in the test net.

TABLE 1

Total Catch Records for Goldeye, *Hiodon alosoides*, Caught During 1974-1976

Sample Area	1974			1975			1976		
	No. Caught	Tagged & Released	Retained	No. Caught	Tagged & Released	Retained	No. Caught	Tagged & Released	Retained
Nipawin, Sask.	882	707	146	462	258	143	-	-	-
Maidstone, Sask.	115	67	48	-	-	-	-	-	-
Maymont, Sask.	-	-	-	47	7	7	-	-	-
River Forks, Sask.	-	-	-				-	-	-
Big Island Sites, Alta.	521	157	302	848	674	286	46	-	46
Devon, Alta.	17	15	2	-	-	-	187	-	141
Subtotals	1535	946	498	1357	939	436	233	-	187

Totals	Caught	Tagged	Retained
	3125	1885	1121

Tag Returns

1974 - 6

1975 - 48

1976 - 5

1977 - 1

TABLE 2

Goldeye Released into the Compound Following Different Means of Capture and Handling

Net caught and released into compound

Tagged	Dead (9 da)	Recaptured in net in compound	Alive at termination
34	15	8	2

total recovery $\frac{25}{34}$ % mortality = 44.1

Line caught and released into compound

Tagged	Dead (9 da)	Alive at termination
25	1	13

total recovery $\frac{14}{25}$ % mortality = 4.0

Line caught and released into compound with no handling

No. released	Dead (9 da)	Alive at termination
11	2	6

total recovery $\frac{8}{11}$ % mortaility = 18.0

Total line caught mortality = $\frac{3}{36}$

= 8.3%

The tagging program in 1975 resulted in a considerable increase in returns (48). Setting aside individual examples for the moment, it appears that goldeye consistently move from one area to the next as it was only on very rare occasions that a tagged goldeye was recaptured within 24 hrs at the site of release (1/60). It was, however, not unusual to recapture tagged goldeye at the release site 1 week to 2 months later (16/60).

In Alberta, direction of movement appeared to be related to the month of tagging (Table 3). In spring and early summer the tagged goldeye moved upstream while in later summer movement was downstream (chi-squared 12.69, $P < 0.001$) (Figure 6A).

Two goldeye tagged in Alberta moved the furthest downstream (approx. 2000 km). One was recaptured 15 days after release at the River Forks and the other was recaptured in the South Saskatchewan River the following spring.

As the summer of 1975 progressed, tags were returned successively farther upstream from Nipawin (Table 3). The last upstream tag was returned in September, 1975, approximately 800 km upstream of Nipawin. Of the several goldeye which moved downsteam from Nipawin, 2 managed to traverse the turbines of the power plant at Squaw Rapids Dam. One was recaptured at Cumberland House the same summer and the second was recaptured at the Pas in Manitoba the following summer (Figure 6B).

The sex of the fish did not appear to have any bearing on the direction or distances move in anyone year.

Figure 7 illustrates the frequency distribution of goldeye sampled in Alberta in 1974-1975. Goldeye under 250 g were not found in Edmonton. Approximately 90% of goldeye exceeding 600 g were female. The population of goldeye throughout the summer differed only in numbers caught and not in distribution (Figure 7).

TABLE 3

Tag Returns from Edmonton Area and Nipawin - Tobin Lake Area

1974-1975

Edmonton Area 1975-1976-1977

Direction of movement	Month Tag Returned			
	May	June	July	August
up	1	7	-	-
none	1	7	5	3
down	-	2	5	7

Nipawin-Tobin Lake Area 1974-1975

up	-	2	2	4
none	1	-	-	-
down	-	-	2	3

Goldeye Sample Sites in Alberta - 1975

indicating location and date of tag release
and location and date of the tag return.

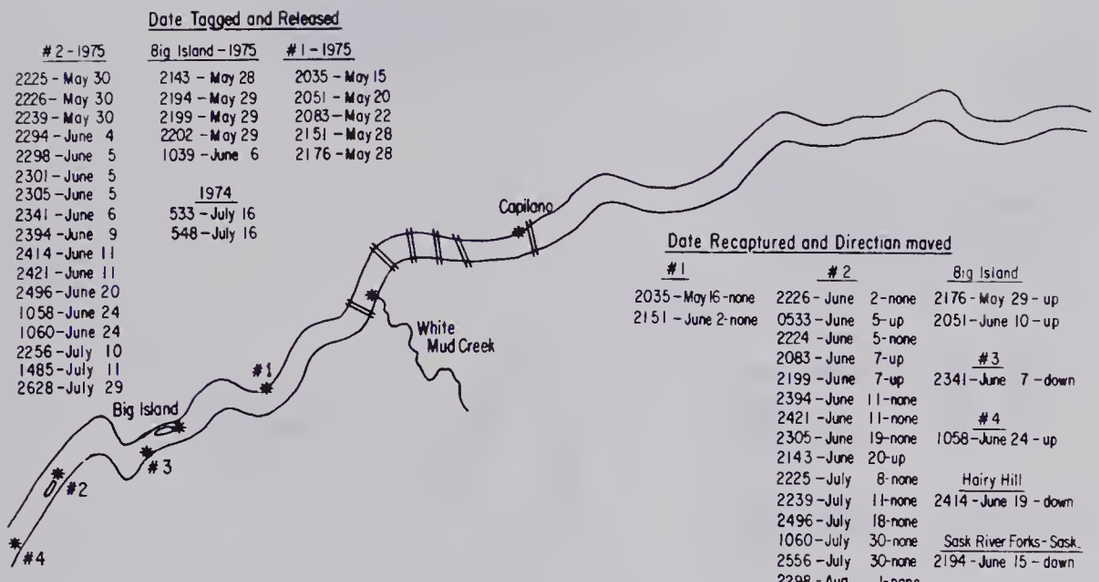

Figure 6A Tag Returns from Alberta 1975

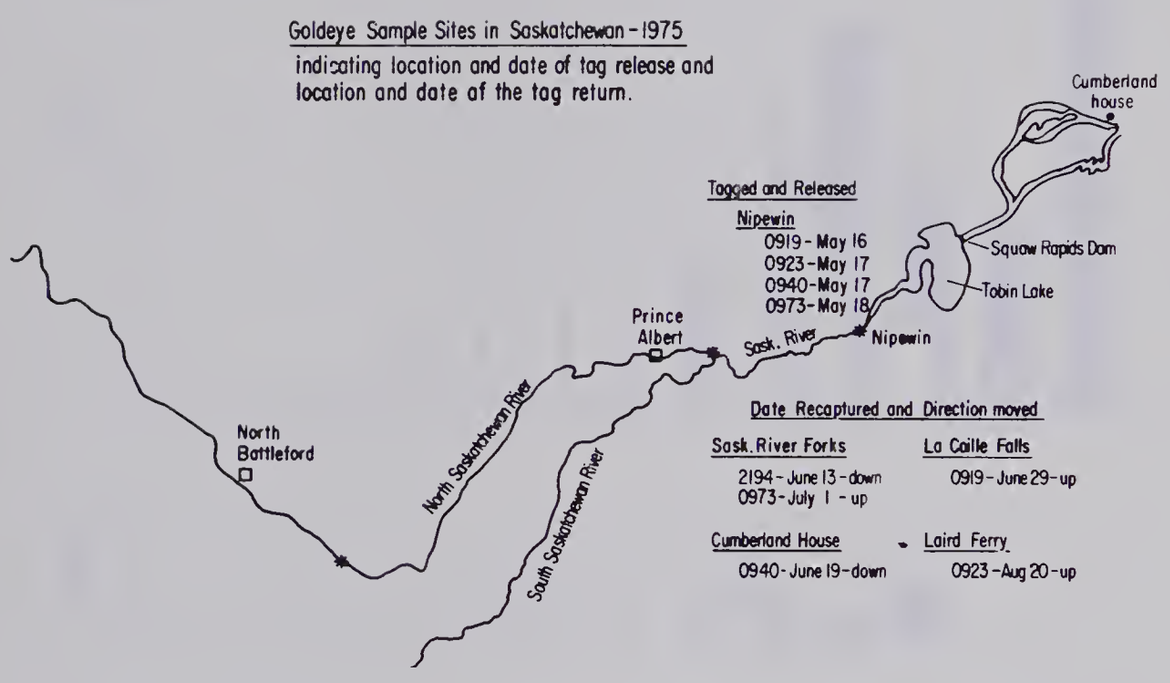

Figure 6B Tag Returns from Saskatchewan 1975

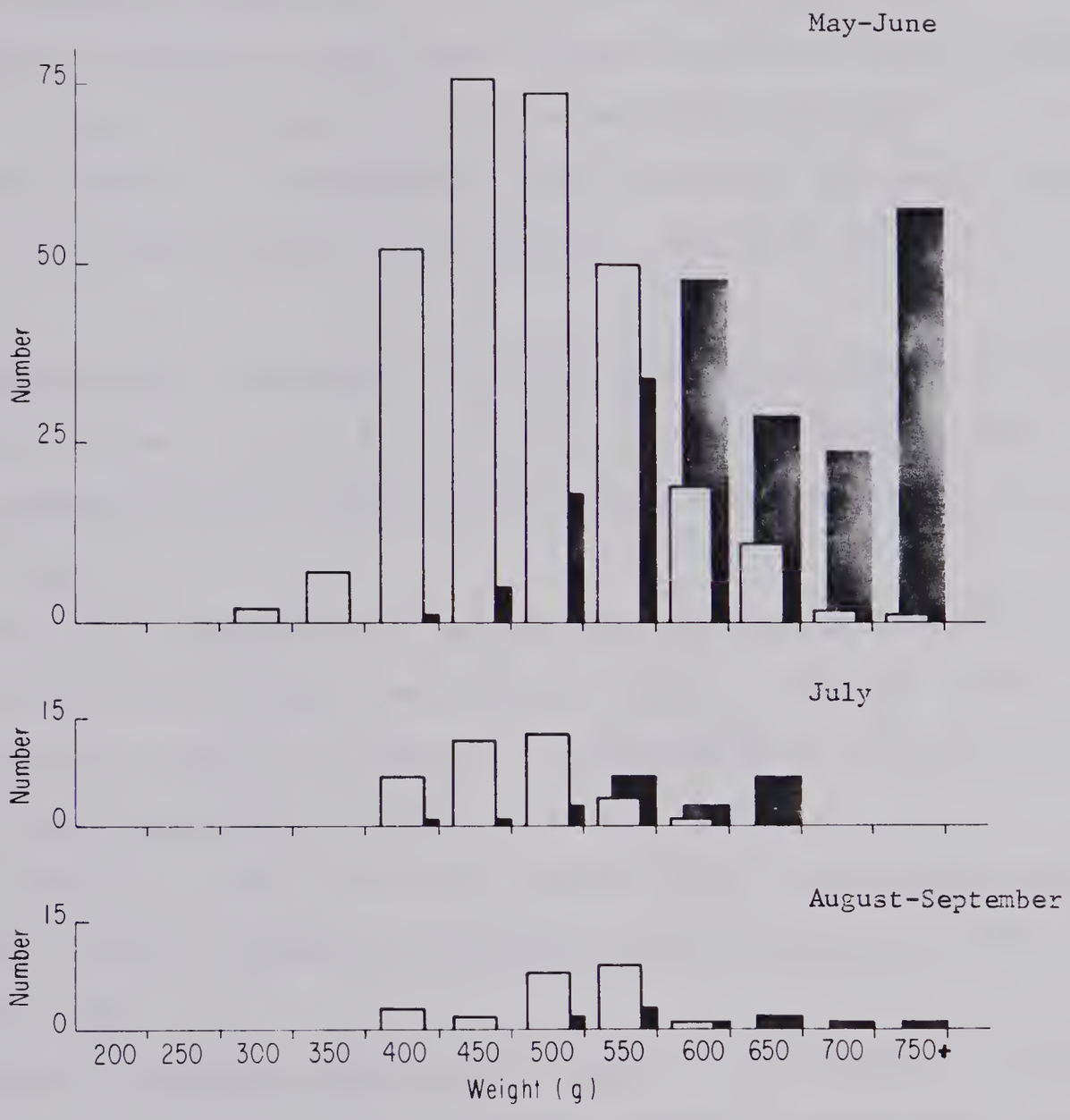

Figure 7 Frequency Distribution of Goldeye at Edmonton, Alberta 1974-1976

These distributions are not significantly different ($P > 0.3$) using the X^2 test dividing the population at the median (Steel and Torrie, 1960). The distributions differ significantly ($0.05 > P > 0.025$) using the Wilcoxon two sample test. This difference appears to be entirely due to the limited number of very heavy fish encountered early in the year and is not encountered if fish weighing in excess of 800 g are excluded from the ranking. The significance was calculated between the month of sampling.
Male - open
Female - shaded

Figure 8 illustrates the frequency distribution of goldeye sampled in Nipawin, Saskatchewan in 1974-1975. In May and June the population of goldeye consisted primarily of fish in the 150-350 gm weight class, while in August and September the population consisted primarily of goldeye weighing 100 gm or less. This change in populations was highly significant ($P < 0.001$, figure 8). Throughout the summer all weight classes were represented in the Nipawin sample, with young-of-the-year first appearing in August.

The age-weight relationship for goldeye caught in the Edmonton area for 1974-1976 is shown in figure 9 and table 4. The youngest goldeye caught in the Edmonton area was 4 years old and this was consistent for the three years of the study.

The similar relationship for goldeye sampled at Nipawin in 1974 and 1975 is also shown in figure 9 and table 4. However in this case young-of-the-year were found in late August. All age classes 0-7 were represented in the Saskatchewan River at Nipawin.

In Edmonton in 1974 for the age classes 4, 5 and 6 in which there were sufficient numbers, females were consistently heavier than males of the same age class.

Goldeye spawning was investigated in Alberta in 1975 and 1976. Results indicated that goldeye do spawn in Alberta as females containing eggs, ripe and apparently ready to spawn were found in Alberta during the spawning period. This period commenced shortly after ice breakup and in 1975 encompassed the time period from mid-May to late June while in 1976 the commencement of spawning was delayed to the first week in June and appeared to terminate in the middle of July. Spawning was determined by the condition and color of the eggs within the female. Females were found with the body cavity completely full of ripe eggs to females with a few eggs in the

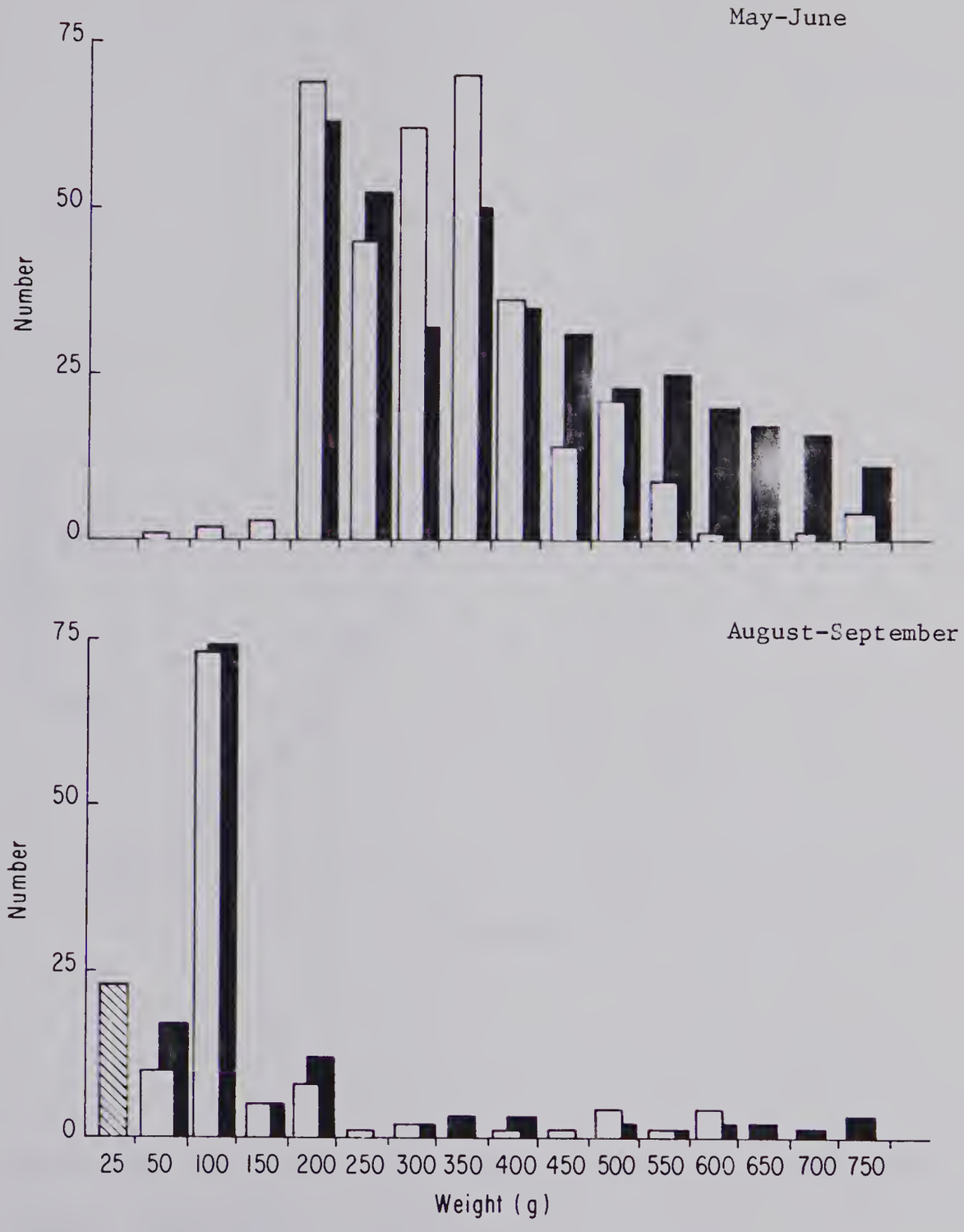

Figure 8 Frequency Distribution of Goldeye at Nipawin, Saskatchewan 1974-1975

These distributions differ significantly ($P < 0.0001$) using both the X^2 test dividing the population at the median (Steel and Torrie, 1960) and the Wilcoxon two sample test corrected for large sample size (Sokal & Rohlf, 1969). The significance was calculated between the month of sampling.
Male - open
Female - shaded
Immature - stippled

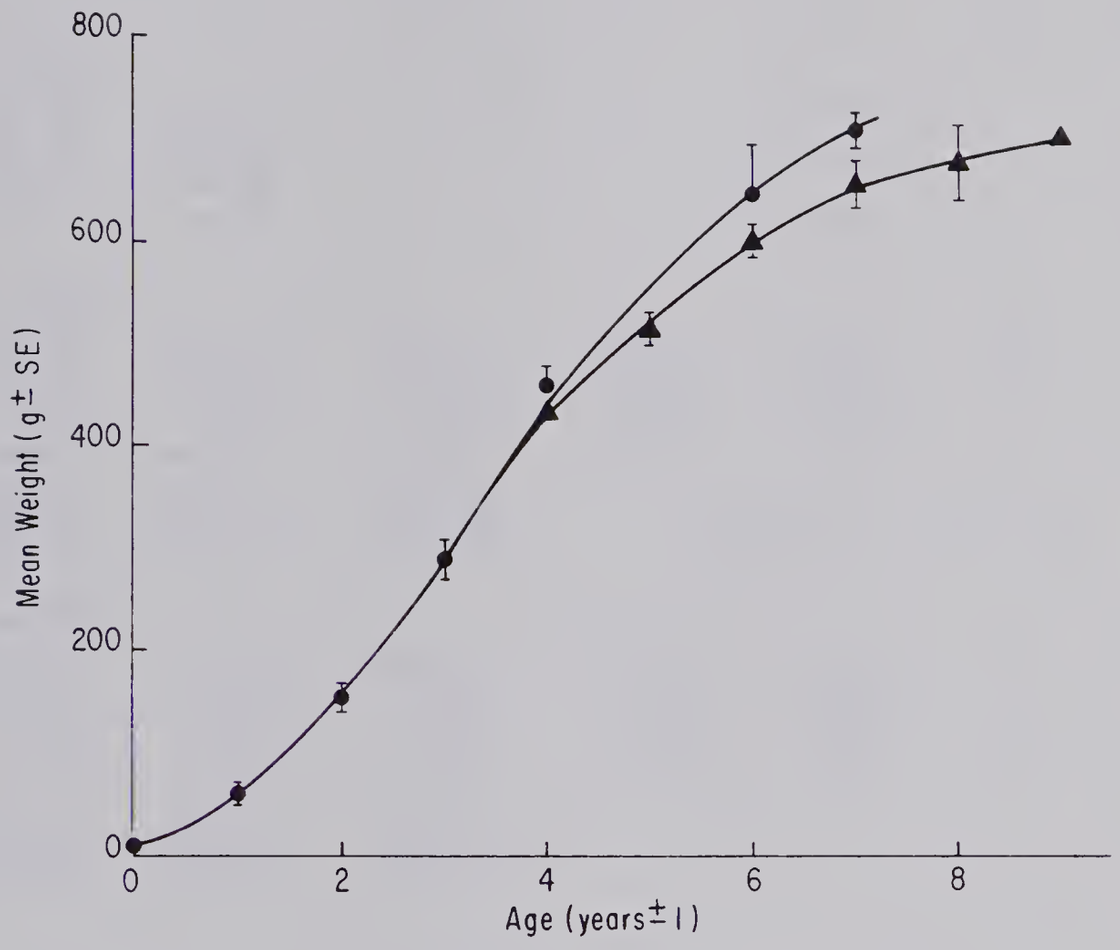

Figure 9 Age-Weight Relationships for Goldeye from Edmonton, Alberta and Nipawin, Saskatchewan 1974-1975

Edmonton ▲
Nipawin ●

TABLE 4

Age-Weight Relationships for Goldeye, *Hiodon alosoides*, sampled at Edmonton, Alberta and Nipawin, Saskatchewan (age-years weight-grams)

Edmonton

Age	4	5	6	7	8	9	10
1974 (weight)							
$\bar{x}$	451.50	486.75	-	-	761.0		
SD	21.92	54.08					
SE	15.5	27.04					
	n=2	n=4			n=1		
1975 (weight)							
$\bar{x}$	432.28	513.00	600.83	651.00	674.5	702	
SD	51.53	60.77	61.83	51.90	43.13		
SE	6.39	5.88	10.31	23.23	30.50		
	n=65	n=107	n=36	n=5	n=2	n=1	
♂ =	429.07	501.95	560.36				
♀ =	448.69	529.25*	618.23*				
1976 (weight)							
$\bar{x}$	420.13	496.02	557.71	629.00	652.60	613	754
SD	35.16	47.04	50.86	44.17	90.11		
SE	6.31	7.09	10.38	13.97	40.30		
	n=31	n=44	n=24	n=10	n=5	n=1	n=1
♂	416.15	500.11	559.82				
♀	450.80	487.18	552.07				

* significant $p < 0.005$

Nipawin

Age	0	1	2	3	4	5	6	7
1974 (weight)								
$\bar{x}$	8.2	59.3	154.42	292.21	459.22	504.67	648.67	715.50
SD	0.75	9.70	27.69	54.38	55.13	56.39	83.34	17.68
SE	0.31	2.10	11.30	14.04	18.38	18.80	48.12	12.50
	n=6	n=22	n=13	n=14	n=9	n=9	n=2	n=2
1975 (weight)								
$\bar{x}$	8.0	60.2	116	322.33	407.40	491.00	-	703
SD	0.61	8.65	-	58.70	61.91	49.40		-
SE	0.29	2.90	-	33.93	27.69	24.70		-
	n=12	n=31	n=1	n=3	n=5	n=4		n=1

process of reabsorption, present in the body cavity and the subsequent year's gonad already developing. In fact females with a body cavity full of ripe eggs were also found to contain a gonad showing signs of developing the subsequent year's eggs. In ripe females the eggs were consistently 20% to the total body weight. Egg development after spawning was rapid and by October of the same year the gonad weight of females had reached 12-15% of total body weight (figure 10). Ovarian weight appeared to be maintained around 15% throughout the winter and in the spring (May) a final 5% was added just prior to spawning.

In early spring (May), at Nipawin, very few females were found to contain ripe ovaries and it was not until late August that significant numbers of females containing mature ovaries began to appear.

Eggs which were expelled from the body cavity singly or in pairs (as determined by stripping) were semi-buoyant and floated singly. Attempts at hatching eggs which had been fertilized in a petri dish proved completely unsuccessful. Free floating eggs following natural spawning were neither observed nor collected. The process of spawning was similarly not observed either due to the state of the river (turbid) or the time of day during which it occurred (night).

Spawning condition of males proved to be considerably more difficult to determine. Ripe males produced approximately 1 cc of milt upon stripping. Development of the testis was not as dramatic as that exhibited by the ovary. No morphological changes were observed to occur during the summer and fall months. In early May the testis became more vascular followed by a slight color change to an opaque white. This color change appeared initially in the posterior region of the testis and was associated with a slight swelling and increased convolution of the testis. As spawning approached, one third to one half of the total length of the testis was

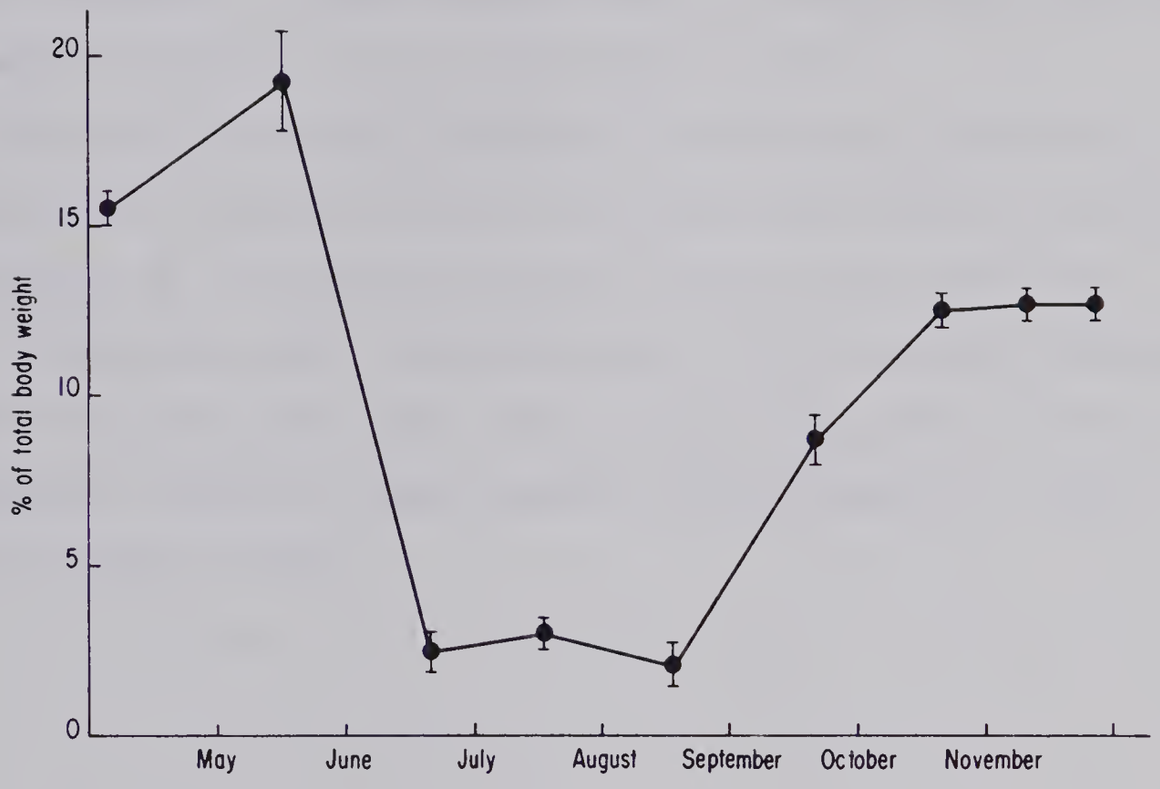

Figure 10 Ovary development in Female Goldeneye in Alberta 1975-1976 (% ± SE)

swollen and convoluted. There was also a slight swelling of the anterior base of the anal fin in the vicinity of the urogenital opening during the spawning period. Overall weight increase of the ripe testis was < 1% of total body weight. It should be noted that the changes noted in the testis at spawning time can, at best, be described as subtle.

Following ice breakup at Edmonton in the spring and throughout the spawning period there was a dramatic increase in the catch per unit effort as evidenced by the frequency diagrams for May and June (figure 8).

Attempts were made to determine possible cues to spawning. Parameters monitored included water level (velocity) water temperature, turbidity and photoperiod. However no single parameter was determined as a single cue for goldeye to spawn.

DISCUSSION

Field studies conducted on goldeye in Alberta and Saskatchewan have revealed a number of facts concerning the status of goldeye in the Saskatchewan River system. Goldeye appear to be in a state of constant movement as evidenced by the lack of tag recaptures, within 24 or 48 hrs after release. Recapture at the release site did occur after 2 months and in a couple of cases from one year to the next. Goldeye appear capable of covering vast distances in relatively short periods of time. In the Edmonton area, the tag return data suggests a directional movement related to the time of year; upstream in May and June, downstream in July and August, while tag return data for goldeye caught at Nipawin suggests that an upstream movement had occurred as the summer progressed.

Since goldeye do spawn in this province but the youngest fish found here is in the 4 yr old class, the success of the spawning must be questioned However certain other characteristics must also be considered such as the floating, single semi-buoyant egg. Because of this egg, the success of upstream spawning will, in all probability be manifested by young-of-the-year being found downstream of the spawning areas.

It is quite possible that the population of goldeye in Alberta is maintained by immigration from a downstream area.

Whether the goldeye population in Alberta is a unique, resident population or a dynamic one encompassing two or maybe three provinces is the subject of considerable speculation and as such the whole goldeye population dynamics in Alberta will be dealt with in considerable detail in chapter 5, especially as it pertains to mercury contamination.

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CHAPTER III

MERCURY LEVELS IN FISH FAUNA FROM THE SASKATCHEWAN RIVER SYSTEM IN ALBERTA AND SASKATCHEWAN

INTRODUCTION

This chapter will deal with the mercury levels found in the fish fauna of the Saskatchewan River system in Alberta and Saskatchewan.

The serious mercury contamination of the Saskatchewan River, as discussed in chapter 1, was traced to the chlor-alkali plant, located at Saskatoon, which released its effluent into the South Saskatchewan River (Sumner et al., 1970).

In an attempt to aid in the understanding of the geodistribution of the mercury which was being introduced to the aquatic environment at Saskatoon, a brief review of the geography of the system will be presented.

The North Saskatchewan River has its headwaters arising on the east slope of the Rocky Mountains (52°08' N, 117°12' W) as a result of melt water from the Saskatchewan Glacier, a tongue of the Columbia Ice Fields (Tebby, 1974). The North Saskatchewan River is part of the Lake Winnipeg drainage system. It is a large turbid, turbulent river with a total length in Alberta of 3,500 km and a drainage area of 93,700 km (Paterson, 1966). The river basin is covered by glacial drift, which being easily eroded, results in steep banks on the outside of bends and gravel flood plains on the inside (Paterson & Nursall, 1975). Where the river flow is rapid, the substrate consists of rubble and gravel, while in the slower moving portions of the river, the substrate is composed of soft mud and sand (Paterson & Nursall, 1975).

The North Saskatchewan River flows eastward into Saskatchewan, where it joins with the South Saskatchewan River, approx. 48 km downstream of Prince Albert to form the Saskatchewan River (figure 1).

Prior to 1963 the Saskatchewan River flowed unobstructed into the Lake Winnipeg system in Manitoba. However in 1964 the Squaw Rapids Dam was constructed on the Saskatchewan River, upstream of Cumberland House; the resulting reservoir was called Tobin Lake.

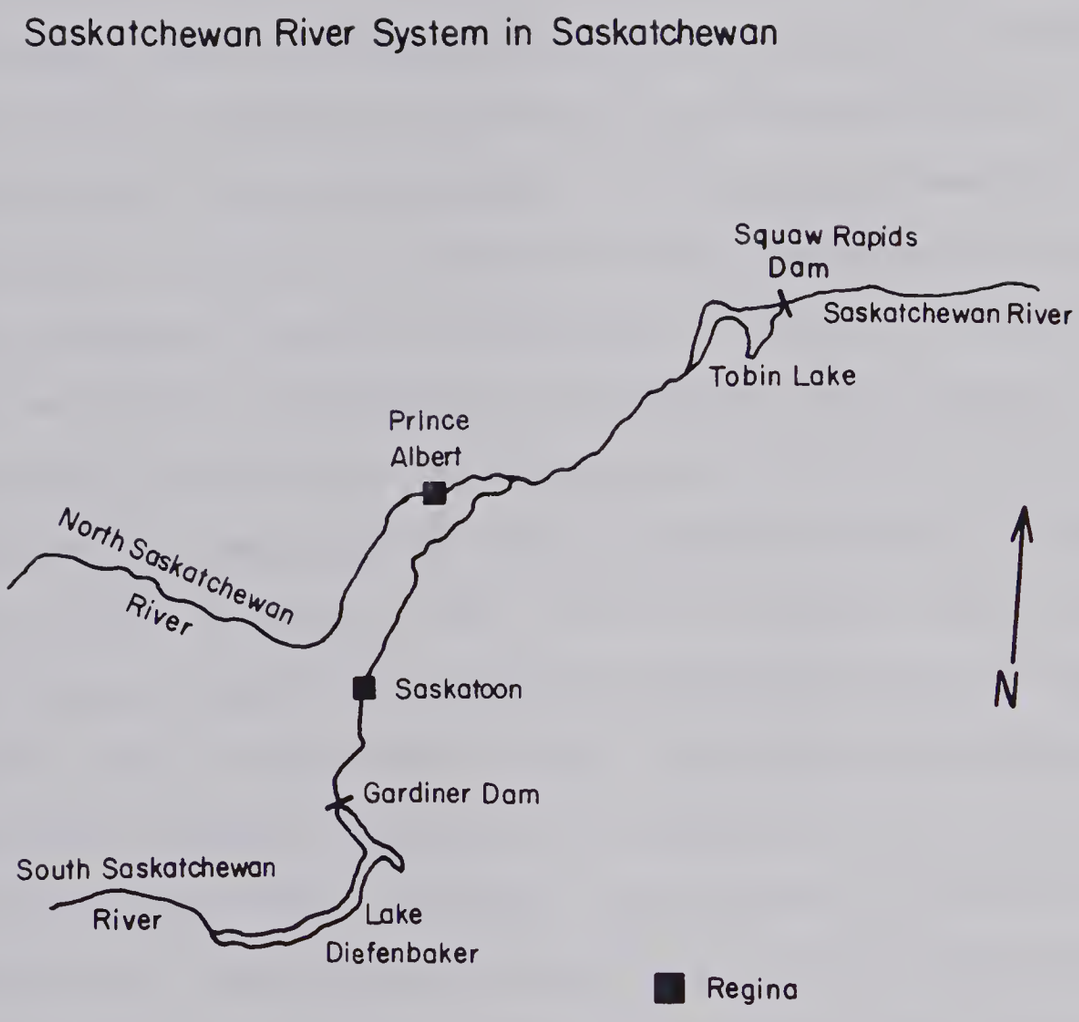

Figure 1 Saskatchewan River System

The North Saskatchewan River has also been subjected to water control and hydroelectric projects which have had marked effects on the physical state of the river. There are two hydroelectric dams situated on the North Saskatchewan River, both located upstream of Edmonton. The Brazeau Dam was constructed in 1963 while the Big Horn Dam went on stream in 1973. The primary function of these dams has been the production of hydroelectric power, however regulation of water flow has also been achieved. Because of its origin in the Rocky Mountains, the North Saskatchewan River and its tributaries are subject to substantial seasonal variations in flow rate. This is especially the case in spring, due to the run-off of melting snow from the winter's accumulation of precipitation. Also, because the principal source of water is from precipitation, the flow rate of the water under the ice cover during the winter is markedly reduced. Therefore there exist periods of marginal flow (< 500 cfs at Edmonton) during the winter and periods of peak flow (> 50,000 cfs at Edmonton) during the spring runoff. These two dams regulate water flow, thereby diminishing the previous substantial seasonal fluctuations, by releasing water from the reservoirs during the winter which maintains the flow rate at levels in excess of 2000 cfs. This would aid in maintaining an elevated dissolved oxygen content under the ice and facilitate to some extent the dilution and dispersion of any contaminants which might be introduced in effluent outfalls. At spring runoff both reservoirs would be filling and therefore the maximum flow rate could be kept below 20,000 cfs.

Since both dams have been functioning, winter flow rate has been maintained at approximately 2000 cfs and no flooding has occurred during spring runoff. However it should be noted that the previous three winters have had significantly less than average precipitation. One other aspect which is somewhat neglected has been the significant daily fluctuations

in flow rates which have occurred, caused by power requirements and not by environmental design.

As information became available concerning the water systems where mercury contamination existed, several basic concepts became obvious. The greater the organic content of the water (increased by industrial and sanitary sewage effluents) coupled with the increased of the velocity of the water, the further the distance downstream the effects of the contamination could be traced (Hartung & Dinman, 1972, Ottawa River Program, 1972). However, when some impediment to water flow was present, such as a lake or dam or reservoir, thereby causing a reduction in water flow, the carrying capacity of water (in terms of sediment) was drastically reduced. Consequently, in the areas of reduced flow, bottom sediments began to accumulate increasing levels of mercury and the pathway to the food chain was formed.

The extent of the mercury contamination problem which has occurred in Saskatchewan illustrates the typical sequence of events experienced in most contaminated areas. The source of mercury, as previously noted, was traced to the chloralkali plant located at Saskatoon which discharged its effluent into the South Saskatchewan River. Fish, sampled immediately downstream of the effluent outfall, contained mercury levels exceeding 12 ppm, with significant amounts of mercury found in sediments and river water (Wobester et al., 1970). Further downstream the river water and bottom sediments contained mercury levels indicative of low level concentration. However at Tobin Lake, approximately 300 miles downstream of Saskatoon, high levels of contamination began to reappear in fish and sediments (Sumner et al., 1970; Wobester, et al., 1970). In 1971, the Saskatchewan Department of Renewable Resources closed fishing of any kind in the whole Saskatchewan River System. Subsequently, under intense economic pressures (tourism) they reopened sport fishery, but on a fish-for-fun basis and this ruling

was still in effect in 1976.

In terms of mercury concentrations in fish in the contaminated areas of Ontario, Manitoba and Saskatchewan the following relationships have been shown to occur. First there existed a positive correlation between mercury concentration in the fish and the relative position of the fish on the food chain (Hartung & Dinman, 1972). Second, the larger or older the fish, of any given species, the higher the mercury concentration. Further, regardless of the chemical form of mercury upon introduction to the aquatic environment, 80-95% of the mercury detected in the fish was methylated (Jernelov, 1972; Lockhart *et al.*, 1972).

As discussed earlier, Munson & Daniel (1973) reported unusual results concerning mercury levels in fish fauna from the North Saskatchewan River. They reported that goldeye, *Hiodon alosoides*, contained significantly higher mercury levels than other species of fish from the same habitat. Becuase of the documented mercury problem in Saskatchewan and the apparent lack of a significant mercury source in Alberta, these authors suggested that the levels of mercury found in Alberta goldeye could have had their origin in Saskatchewan. The extended ½ life of mercury in fish (Lockhart, *et al.*, 1972) and the migratory behaviour patterns of goldeye have encouraged investigation into the possibility that the mercury found in goldeye may indeed have had its origin in Saskatchewan.

This theory was tested by determining mercury levels in goldeye sampled from a contaminated area in Saskatchewan (Tobin Lake) which was compared to mercury levels in goldeye sampled from a non-contaminated area located a considerable distance upstream (Edmonton).

Mercury levels in other species of fish, which do not migrate vast distances were also examined from both areas. The mercury levels in these fish would be indicative of their respecitive environment.

Tissue/organ distribution of mercury in goldeye from the two areas was also examined. Tissues which possess an accelerated turnover rate, in terms of mercury concentration, might possibly show some variation in relation to recent exposure and hence provide evidence that the Edmonton fish had not recently been exposed to mercury contamination.

MATERIALS AND METHODS

Specimens for mercury analysis were collected in a similar manner and at the same sites as described in Chapter II. The samples, which were to be utilized for mercury analysis, were stored in plastic bags and frozen, where possible, within one hour after capture.

Duplicate tissue samples of dorsal axial white musculature were taken anterior to the dorsal fin. Total mercury analysis was performed on these tissues.

The tissues were dissolved in concentrated H_2SO_4 and concentrated HNO_3, subsequently oxidized by 7% $KMnO_4$ and subsequently reduced by a 1% solution of $SnCl_2$. Analysis for total mercury content on the digested samples was conducted by flameless atomic absorption spectroscopy utilizing a Unicam SP90A specrotrophotometer (Thorpe, 1971; Armstrong & Uthe, 1971; Magos, 1971).

Standard curves using mercuric chloride ($HgCl_2$) were constructed with each set of fish tissue analyzed (figure 2). Appropriate chemical blanks were run simultaneously with the standard curve.

The accuracy of the technique was determined by recovery experiments utilizing $HgCl_2$ and CH_3HgCl and consistently yielded 87-102% recovery.

Orchard leaves, National Bureau of Standards, Standard Reference Material 1571, were also utilized to monitor accuracy. The mercury content of the orchard leaves was determined to be 0.147 ± 0.056 S.D. µg/g, n=24. The reported value was 0.155 ± 0.015 µg/g dry weight.

Precision of the technique was determined by analyzing 10 homogenized samples of white skeletal muscle from the same fish. Precision, as determined by this method, produced a variation between samples of 10% or less.

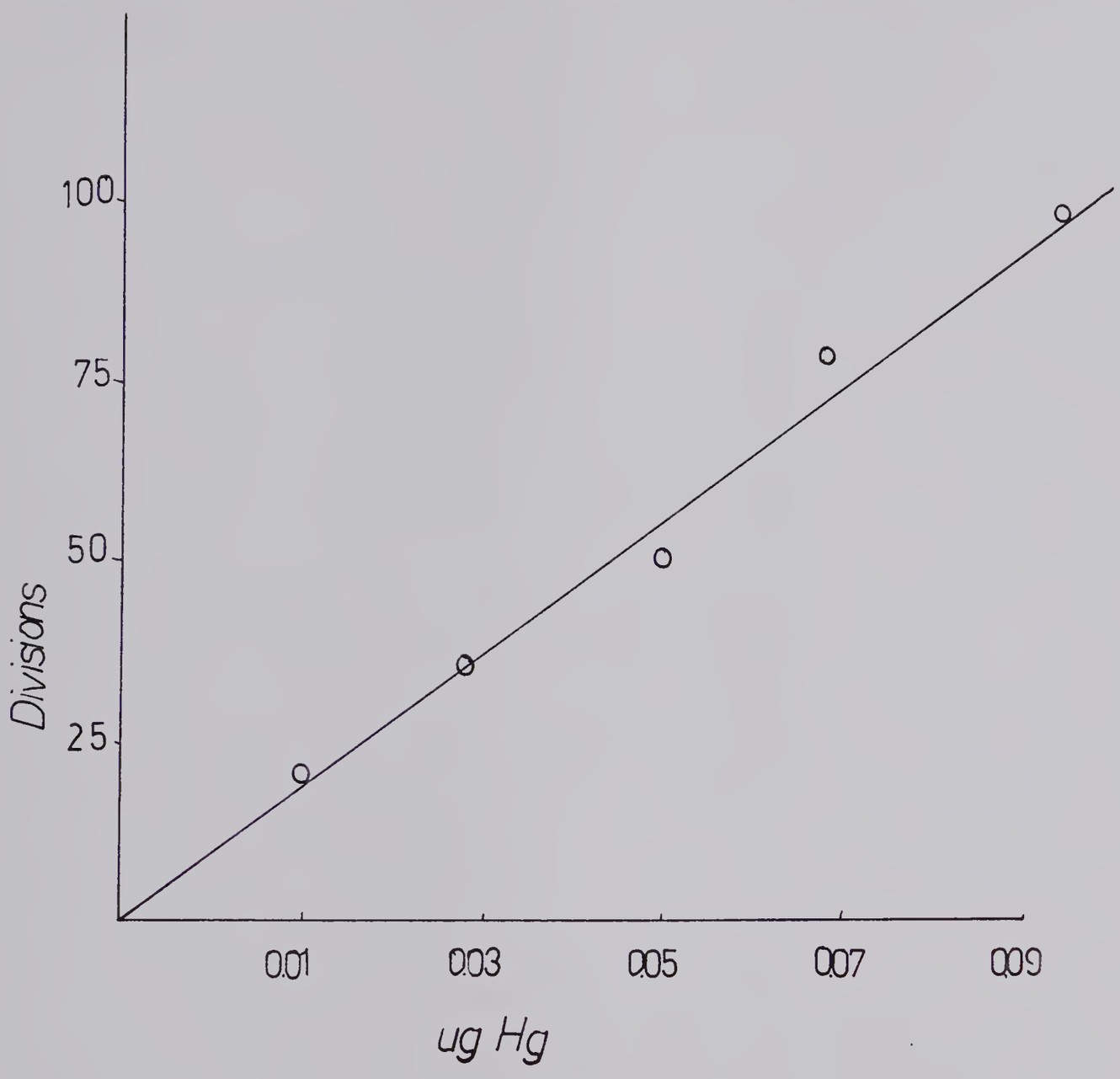

Figure 2 Mercury Standard Curve

Total mercury determinations for all fish samples are expressed as parts per million (ppm), i.e., µg of mercury per gram of fish tissue, wet weight.

RESULTS

The mercury levels in goldeye from Edmonton, Alberta and Nipawin, Saskatchewan are illustrated in figure 3 and table 1. There was no significant difference in mercury levels in goldeye sampled from these two areas for the two years that both areas were tested concurrently. Also, within both areas throughout the test period, there was a decrease in the mean levels of mercury found in the muscle tissue. In Edmonton the decrease in mercury levels was significant from 1973 to 1975 while in Nipawin the decrease was significant from 1974 to 1975.

Mercury levels found in the axial white muscle of walleye, *Stizostedion vitreum*, sampled from Edmonton and Nipawin are presented in figure 4 and table 2. There was a signficant difference between mercury levels in walleye sampled in Nipawin and Edmonton in 1974 but not in 1975. For the two years that this fish was analyzed for total mercury, walleye from Nipawin contained more mercury than walleye from Edmonton. There was a significant drop in mercury levels in walleye from Nipawin from 1974 to 1975. No such significant decrease was observed in walleye sampled at Edmonton from 1973 to 1976.

Samples of white muscle of sauger, *Stizostedion canadense*, which had been collected in both areas, were analyzed for total mercury and the results are depicted in figure 5 and table 3. Sauger sampled at Nipawin had significantly higher mercury levels than sauger sampled from Edmonton in 1974, when the sample size was sufficiently large. There was a significant decrease in the mercury levels in sauger from Nipawin between 1974 and 1975 while no such significant change in mercury levels in sauger from Edmonton was noted over the period 1973-1976. When walleye and sauger of corresponding size were compared, sauger contained significantly higher mercury levels ($p < 0.05$) than walleye in Edmonton. At Nipawin this relationship held but was not significant.

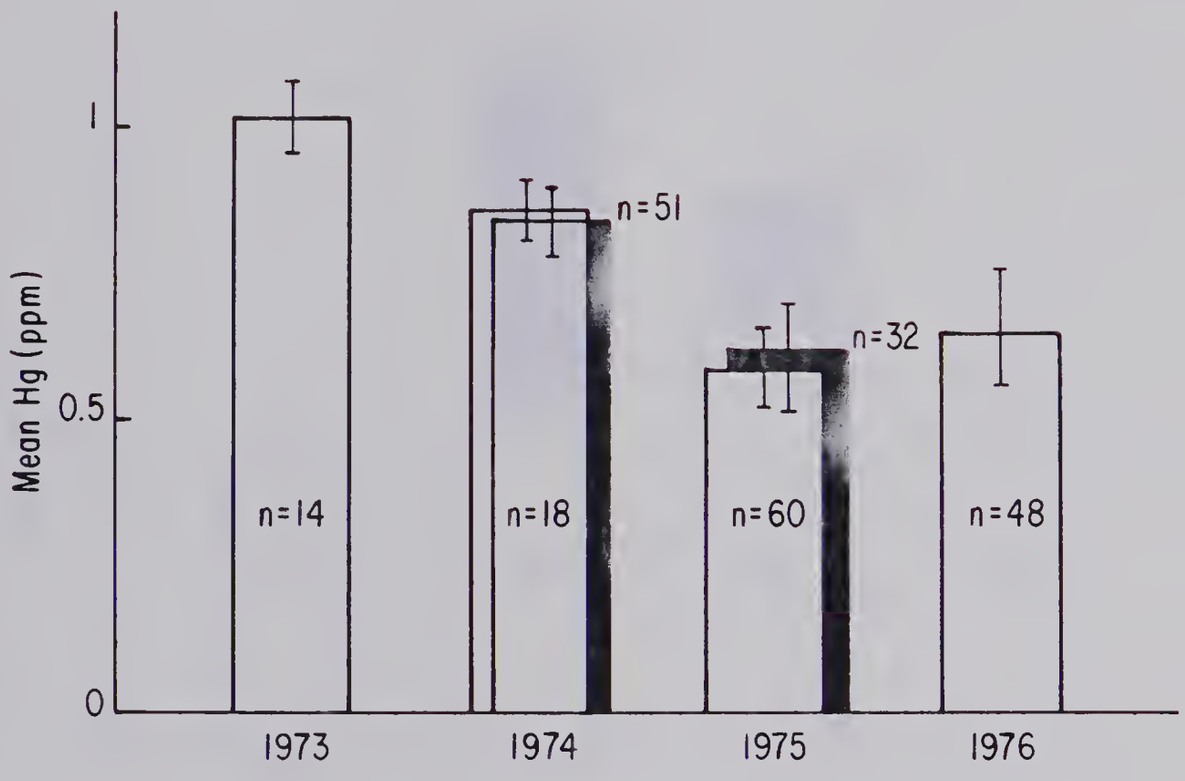

Figure 3 Mercury Levels in Goldeye from Edmonton, Alberta and Nipawin, Saskatchewan 1973-1976. (ppm $\pm$ SE)

Edmonton - open
Nipawin - shaded

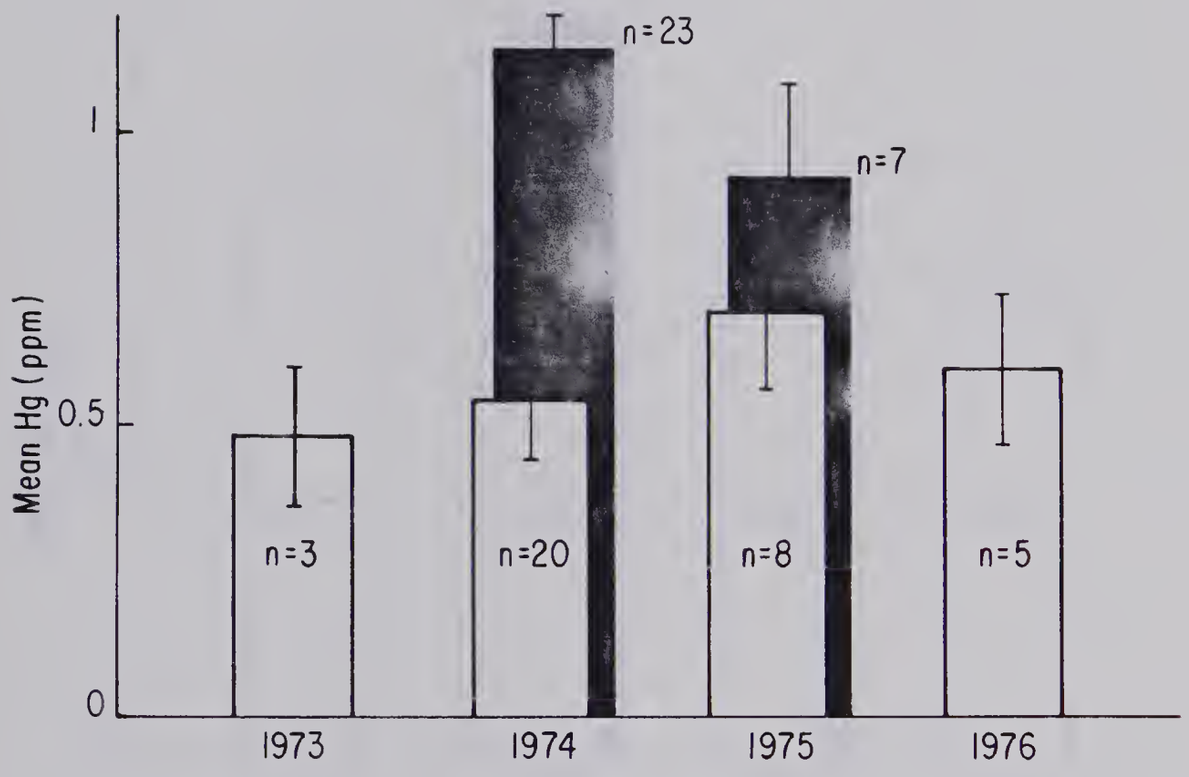

Figure 4 Mercury Levels in Walleye from Edmonton, Alberta and Nipawin, Saskatchewan 1973-1976. (ppm $\pm$ SE)

Edmonton - open
Nipawin - shaded

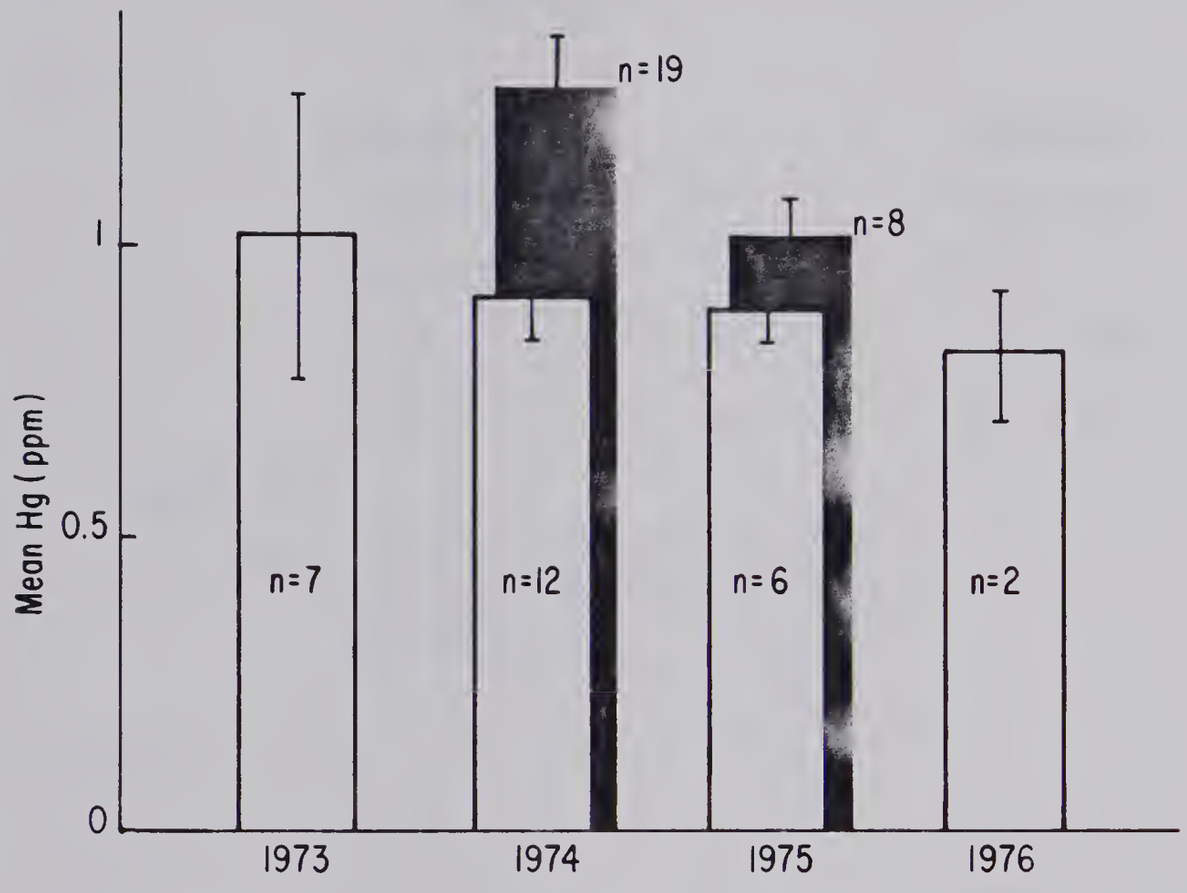

Figure 5 Mercury Levels in Sauger from Edmonton, Alberta and Nipawin, Saskatchewan 1973-1976. (ppm $\pm$ SE)

Edmonton - open
Nipawin - shaded

TABLE 1

Mercury Level in White Muscle from Goldeye, *Hiodon alosoides*, Sampled from Edmonton, Alberta and Nipawin, Saskatchewan 1973-1976

Edmonton

	mean ppm	N	Range ppm
1973	1.02	141	0.34 - 4.40
1974	0.86	18	0.20 - 2.23
1975	0.59	76	0.16 - 2.00
1976	0.65	48	0.17 - 1.90

Significance*

1973-1974 - Drop sig. $p < 0.05$

1974-1975 - Drop sig. $p < 0.05$

1975-1976 - N.S.

Nipawin

1974	0.84	51	0.26 - 2.41
1975	0.62	32	0.24 - 2.01

Significance

1974-1975 - Drop sig. $p < 0.05$

Edmonton - Nipawin Significance

1974 - N.S.

1975 - N.S.

* Students t-test for unpaired samples.

TABLE 2

Mercury Levels in White Muscle from Walleye, *Stizostedion vitreum*, Sampled from Edmonton, Alberta and Nipawin, Saskatchewan 1973-1976

Edmonton

	mean ppm	N	Range ppm
1973	0.48	3	0.21 - 0.69
1974	0.34	20	0.17 - 0.88
1975	0.69	8	0.21 - 0.98
1976	0.59	5	0.16 - 0.89

Significance

1973-1974 - N.S.
1974-1975 - N.S.
1975-1976 - N.S.

Nipawin

1974	1.14	23	0.67 - 2.01
1975	0.92	7	0.41 - 1.47

Significance

1974-1975 - Drop sig. $p < 0.05$

Edmonton - Nipawin Significance

1974 - sig $p < 0.05$
1975 - N.S.

TABLE 3

Mercury Levels in White Muscle from Sauger, *Stizostedion canadense*
Sampled from Edmonton, Alberta and Nipawin, Saskatchewan
1973-1976

Edmonton

	mean ppm	N	Range ppm
1973	1.02	2	0.92 - 1.12
1974	0.91	12	0.84 - 1.32
1975	0.89	6	0.90 - 1.19
1976	0.81	2	0.74 - 0.88

Significance

1973-1974 - N.S.
1974-1975 - N.S.
1975-1976 - N.S.

Nipawin

1974	1.27	19	0.80 - 2.21
1975	1.02	8	0.62 - 1.48

Significance

1974-1975 - Drop sig. $p < 0.05$

Edmonton - Nipawin Significance

1974 - sig. $p < 0.05$
1975 - N.S.

At this juncture it should be noted that no young-of-the-year of any species analyzed showed any significant increase in mercury levels beyond that which would be expected for background levels (0.14 - 0.27 ppm).

Where goldeye, walleye and sauger were caught in sufficient numbers and were analyzed for mercury content, and values compared, the data were restricted to similar age classes. That is to say all the data which were presented for walleye and sauger included only fish in excess of three years old. Although all age classes of goldeye were represented in the Nipawin sample only those fish in excess of three years of age were included in the comparative data. The Edmonton sample of goldeye was restricted to 4 year old or older fish.

Figure 6 and table 4 illustrate the mercury levels found in pike, *Esox lucius*, in the Edmonton area. No significant change in the mercury levels in pike was observed from 1973-1976. Pike from Nipawin were not analyzed for mercury levels.

Four species of suckers, white sucker (*Catostomus commersoni*) long-nose sucker (*Catostomus catostomus*) northern and silver redhorse (*Moxostoma* spp) from both Edmonton and Nipawin were sampled and analyzed for total mercury levels. Although the sample sizes were admittedly small, no significant differences were detected between areas or from one year to the next (figure 7 and table 5).

Goldeye was the sole species of fish where mercury levels were correlated statistically with weight and age. There was a positive correlation ($r=0.922$, $p < 0.01$) between the total weight of the goldeye and mercury concentration (figure 8). There also existed a positive correlation ($r=0.911$, $p < 0.01$) between the age of the goldeye and the mercury concentration (figure 9). This analysis was conducted only on the Nipawin sample because it was only in this sample that the younger age classes, and therefore weight classes, were represented.

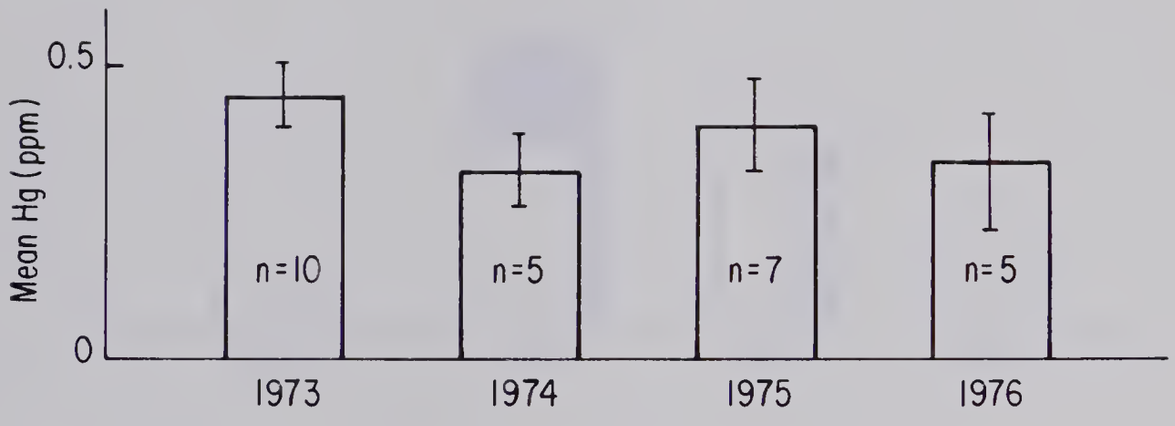

Figure 6 Mercury Levels in Northern Pike from Edmonton, Alberta 1975 - 1976. (ppm ± SE)

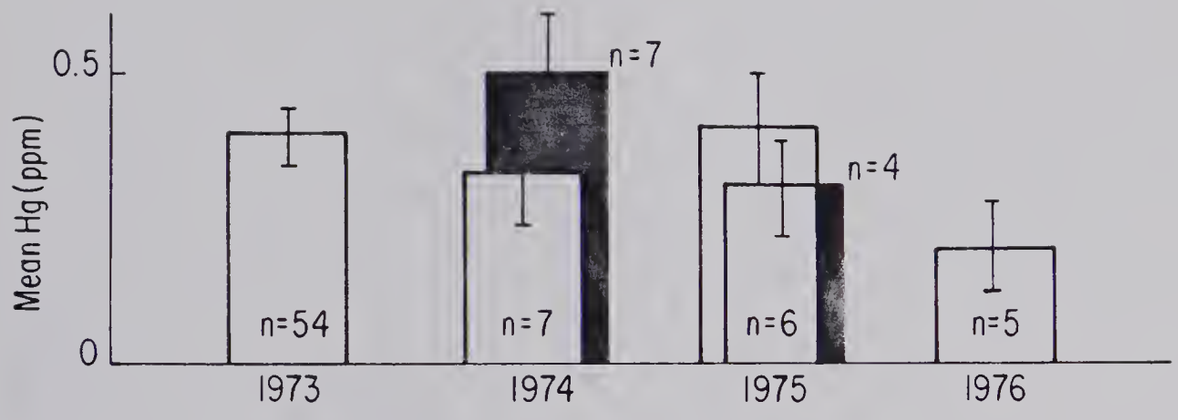

Figure 7 Mercury Levels in Four Species of Sucker; Longnose, White, Northern and Silver Redhorse, from Edmonton, Alberta and Nipawin, Saskatchewan 1973-1976. (ppm $\pm$ SE)

Edmonton - open
Nipawin - shaded

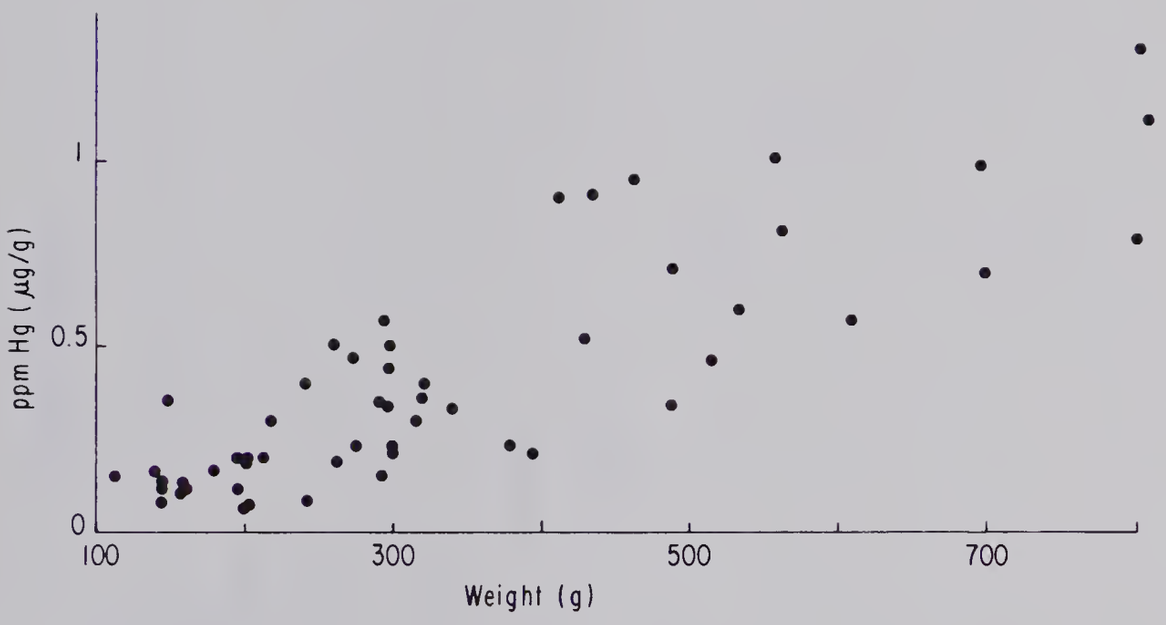

Figure 8 Mercury Concentration vs Weight of Goldeye from Nipawin, Saskatchewan 1974

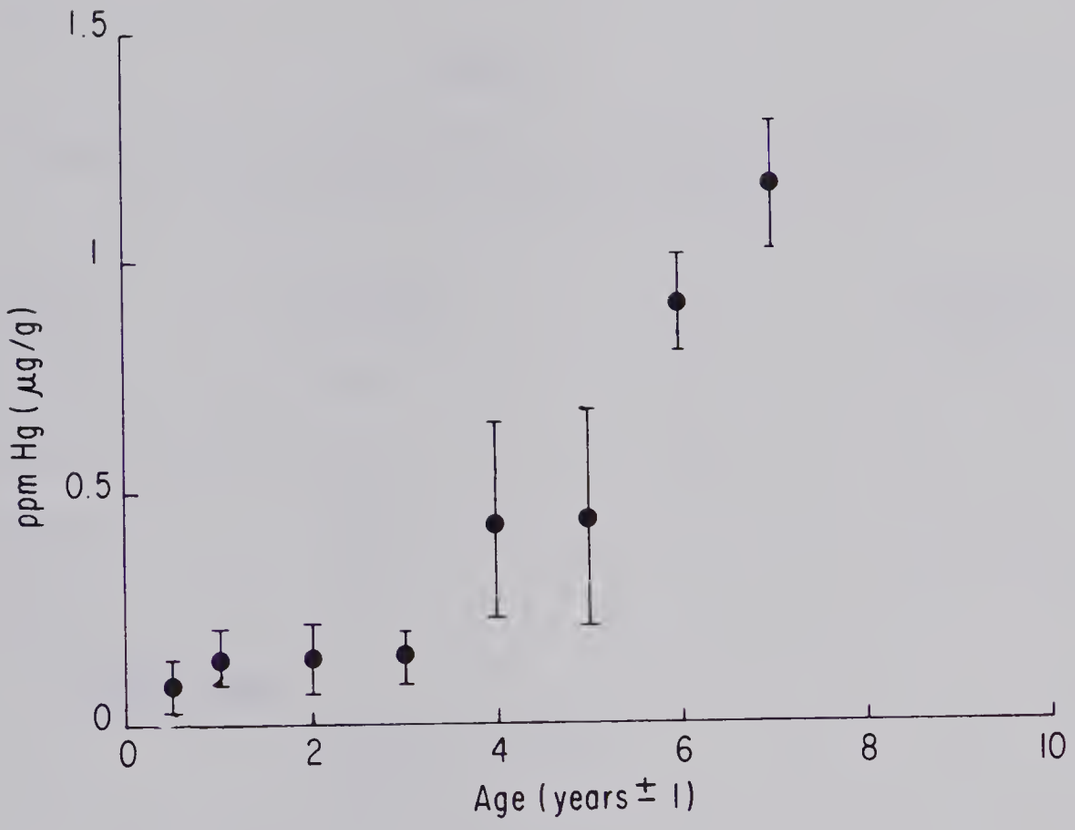

Figure 9 Mercury Concentration vs Age of Goldeye from Nipawin, Saskatchewan 1974. (ppm $\pm$ SE)

TABLE 4

Mercury Levels in White Muscle of Pike, *Esox lucius*, from Edmonton, Alberta, 1973-1976

Year	mean ppm	N	range ppm
1973	0.45	10	0.17 - 0.72
1974	0.32	5	0.22 - 0.69
1975	0.40	7	0.19 - 0.72
1976	0.34	5	0.24 - 0.61

Significance

1973-1974 - N.S.

1974-1975 - N.S.

1975-1976 - N.S.

TABLE 5

Mercury Levels in White Muscle of Four Species of Sucker; White Sucker, *Catostomus commersoni*, Longnose Sucker, *Catostomus catostomus*, Northern and Silver Redhorse, *Moxostoma* spp., Sampled from Edmonton, Alberta and Nipawin, Saskatchewan

Edmonton

	mean ppm	N	range ppm
1973	0.40	54	0.29 - 0.48
1974	0.33	7	0.22 - 0.44
1975	0.41	6	0.17 - 0.51
1976	0.19	5	0.12 - 0.36

Significance

1973-1975 - N.S.

Nipawin

1974	0.49	7	0.21 - 0.52
1975	0.31	4	0.27 - 0.43

Significance

1974-1975 - N.S.

Edmonton - Nipawin Significance

1974 - N.S.

1975 - N.S.

Tissue/organ distribution of mercury in the natural goldeye population was examined in 1974 for both Edmonton and Nipawin. Table 6 illustrates that in both areas the lens of the eye contained the highest levels of mercury followed closely by the white muscle. However, the similarity between the mercury levels in goldeye sampled from Alberta and Saskatchewan began to differ when the remainder of the tissues were examined. In Alberta there was a dramatic drop to the next tissue, kidney, followed by very low levels in the liver, red muscle, and gonad. Goldeye sampled in Saskatchewan, on the other hand, had high levels in the kidney and liver followed by slightly decreased levels in the gonad. Statistical analysis revealed that the difference in mercury levels noted between the kidney, liver and gonad from the Edmonton and Nipawin samples was significant at $p < 0.05$.

TABLE 6

Mercury Levels in Various Tissues/Organs from Goldeye, *Hiodon alosoides*, Sampled from Edmonton, Alberta and Nipawin, Saskatchewan in 1974

Tissue	Edmonton n=18		Nipawin n=45	
	mean ppm	range	mean ppm	range
Lens	1.01	0.62 - 2.40	1.26	0.87 - 2.61
White Muscle	0.86	0.20 - 2.23	0.84	0.26 - 2.41
Kidney	0.27	0.08 - 0.36	0.78	0.41 - 0.99
Liver	0.19	0.00 - 0.34	0.61	0.27 - 0.87
Red Muscle	0.08	0.00 - 0.19	---	---
Gonad (Female)	0.00	---	0.29	0.07 - 0.44

DISCUSSION

Mercury levels in three species of fish, goldeye, walleye and sauger were investigated in considerable detail from two separate areas, Edmonton, Alberta and Nipawin, Saskatchewan.

Goldeye were chosen because of the high levels of mercury found in these fish in the North Saskatchewan River in Alberta in the 1973 survey. Walleye and sauger were chosen because they are solely piscivorous and therefore occupy a high position on the food chain. They also do not have a history of migrating vast distances (Scott & Crossman, 1973) and are relatively abundant in both sampling areas. These two species of fish are strikingly similar in appearance but possess different growth rates (Scott & Crossman, 1973). Walleye grow, on the average, at a rate which is three times that exhibited by sauger, given the same environmental conditions and food availability (de Freitas et al, 1972). Therefore, in areas where mercury contamination is present in the aquatic environment, walleye should possess significantly less mercury than that found in sauger of the same age class. This is primarily due to the phenomenon of growth dilution.

In both areas from which sauger and walleye were sampled and were of corresponding size, sauger contained higher mercury levels than walleye. This difference was significant at Edmonton but not at Nipawin.

Both walleye and sauger sampled at Nipawin contained higher mercury levels than their counterparts from Alberta. This finding agrees with the suggestion that the closer the fish is sampled to the source of contamination the higher the levels of that contaminant in that particular animal will be. This is also assuming two separate populations, one in each area, with no physical contact with one another.

Goldeye also sampled from Nipawin and occupying a mid-position on the food chain showed relatively high levels of mercury although significantly less than ($p < 0.05$) that found in walleye and sauger.

Therefore the data from Nipawin indicates that the fish population in a contaminated area adheres to the concept of food chain magnification. There are, however, more important factors which contribute to the mercury levels in fish from any given area, such as age, growth rate and behavorial characteristics (de Freitas et al, 1972).

The mercury contamination in Saskatchewan had its source at Saskatoon from the chlor-alkali plant which discharged its effluent into the South Saskatchewan River. In 1972 the Federal Department of the Environment (presently Environment Canada) restricted the levels of mercury permitted in the effluent from chlor-alkali plants to less than three grams per day. Because of the dynamics of mercurial compounds in bottom sediments and aquatic ecosystems, it was originally determined that decades would elapse before the surface waters could naturally disperse the mercury which had previously been introduced in such vast quantities (30 kg/da) over a considerable period of time. It was also believed that animals in or associated with contaminated water systems would similarily display a very slow recovery to background levels.

In the present study there was a decrease in the mercury levels of virtually all of the fish species studied from Nipawin, Saskatchewan. Data received from the Department of Renewable Resources of Saskatchewan (F.M. Atton, pers comm) also indicate that a significant decrease in mercury levels in fish species has occurred from 1973 to 1976. It can only be assumed that this decrease in the mercury levels in fish is directly related to the cessation of mercury contamination from the chlor-alkali plant in Saskatoon. If this indeed proves to be the case

then aquatic ecosystems may possess the ability to recover from mercury contamination much more quickly than originally believed. Our original calculations on the rate and degee to which mercury compounds are mobilized from bottom sediments by bacterial mediated methylation may have been grossly exaggerated.

In Alberta, the original study in 1973 revealed that goldeye contained the highest levels of mercury contamination of all the species tested (Munson & Daniel, 1973). These findings did not follow proposed food chain magnification, in that pike and walleye, above goldeye on the food chain, contained signficantly less mercury ($p < 0.05$). The levels found in goldeye in 1973 were also not consistent with the degree of mercury contamination which existed in the North Saskatchewan River at that time.

The levels of mercury found in all species of fish sampled in Alberta, with the exception of goldeye, maintained at or near those 1973 levels throughout the present study. This data is what would be expected from a static environment in terms of mercury contamination.

Goldeye, however, have shown a consistent and significant decrease in mercury levels from 1973 to 1975. Quantitatively, the mercury levels in goldeye from Alberta were virtually identical to those levels found in goldeye from Nipawin, Saskatchewan for the two years that comparatative studies were conducted. Similarly the decrease that occurred in the mercury levels in Alberta goldeye parallels the observed decrease in the mercury levels found in goldeye sampled at Nipawin.

Since there has not been any observable decrease in mercury levels in other species of fish in Alberta and since the absolute levels of mercury in Alberta goldeye, as well as the observed decrease in levels, parallels the situation in Saskatchewan, it appears that the populations of goldeye in the two provinces are not distinct and separate.

Although goldeye sampled from Alberta and Saskatchewan possessed similar levels of mercury in their white muscle, a closer examination of the mercury in various other tissues revealed an interesting distribution phenomenon possibly related to the levels of mercury present in the immediate environment prior to capture.

Goldeye sampled in Alberta possessed the highest levels of mercury in the lens of the eye followed closely by the white muscle. Mercury levels in the kidney, liver, red muscle and gonad were very low when compared to the white muscle.

Goldeye sampled in Saskatchewan possessed the highest levels in the lens followed by white muscle kidney, liver and gonad. But all tissues, with the exception of the lens and white muscle, possessed significantly higher ($p < 0.05$) levels than was observed in their counterparts from Alberta. The situation found in the various tissues of goldeye from Saskatchewan is indicative of chronic exposure to mercury which, at the time of sampling, was still present to some degree. The fish were actively metabolizing and eliminating the input.

The distribution of mercury in tissues of goldeye from Alberta is indicative of fish which had been exposed to mercury at some time in the past but were presently inhabiting a relatively mercury free environment. In these fish, therefore, it is only those tissues in which mercury has an extended half-life that detectable levels of mercury will occur. Tissue distribution of mercury in goldeye, rather than absolute levels in skeletal muscle, depicts the differences in the degree of mercury contamination in each of the two areas under investigation.

This phenomenon of tissue distribution of mercury, particularly in goldeye, will be examined in considerably greater detail in Chapter IV.

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CHAPTER IV

PHYSIOLOGICAL AND PHARMACOLOGICAL DISTRIBUTION OF MERCURY IN GOLDEYE

INTRODUCTION

Present knowledge governing the origin and dispersion of mercury through the aquatic, terrestrial and atmospheric ecosystems is at an advanced state. It is, therefore not surprising that current attention has been focused on the basic pathways of accumulation, distribution and excretion of mercury at specific trophic levels.

The mercury found in fish from natural populations is approximately 90% in the methylated form (Westöö and Niven, 1967; Johansson *et al.*, 1970), which therefore implies the existence of two possibilities to examine this phenomenon. First, the mercury to which the fish were originally exposed, either dietary or environmental, was already in the methylated form or second, the mercury originated in the inorganic form and was methylated within the body of the fish subsequent to ingestion. In order to properly determine the most probable chemical form of the mercury prior to uptake, several basic parameters must be reviewed. Methylmercury is readily absorbed from the gut and will cross the gill filamental membranes with relative ease and efficiency. Methylmercury exhibits these features because of its single valence, relatively small size and high lipid solubility (McKim *et al.*, 1976). Inorganic mercury on the other hand, is poorly absorbed from the gut and does not readily cross cellular or gill membranes (Hannerez, 1968). Inorganic mercury, when introduced into the aquatic ecosystem is relatively heavy and insoluble and will thus settle to the bottom. It is in this segment of the ecosystem that the existence of bacteria, possessing the ability to methylate the insoluble inorganic mercury, was uncovered by Wood *et al.* (1968). Once methylated, the mercury becomes much more mobile in the aquatic environment due to increased solubility in water, and an affinity for organic and fine sediment particules dissolved or sus-

pended in the water column. Methylmercury may also be introduced directly and will of course experience the same fate. Mercury, present in the methylated form in ambient water, can be directly taken up by fish via absorption through the gills of body (McKim *et al.*, 1976). It has been suggested that direct uptake may account for 50% of the mercury levels in the fish (Jernelöv, 1972). Mercury contamination resulting from direct exposure would therefore not follow food chain magnification theories as discussed in Chapter III. The growth rate of each individual fish becomes a more important factor than trophic level location because of the biomass dilution factor (McKim *et al.* 1976). A fish species possessing an accelerated age-weight relationship would therefore contain lower weight-specific mercury levels. McKim *et al.* (1976) state that the amount of mercury accumulated is directly proportional to the water concentration and, that at a water concentration of 0.03 ug Hg/l in a 100 day exposure fish would possess mercury levels in muscle tissue well in excess of the 0.5 ug/gm level.

Basic parameters such as growth rate and mode of exposure have proved to be as important as location on the food chain (Weisbart, 1973; Dickinson & Krenkel, 1973; McKim *et al.*, 1976). Therefore the actual levels of mercury detected in fish will include mercury originating from direct exposure from a mercury-contaminated environment coupled with mercury which has been indirectly accumulated via the food chain. It must be kept in mind that if mercury is present in the food chain it is also present in the environment.

The statement that the relative position of any particular species on the food chain will be the determining factor in body burden of mercury remain valid but subject to the qualifications mentioned above.

Figure 1 represents an attempt to illustrate, in a flow chart, a summary of data taken from several of the most recent article concerning mercury uptake, distribution, and excretion in fish based on observation

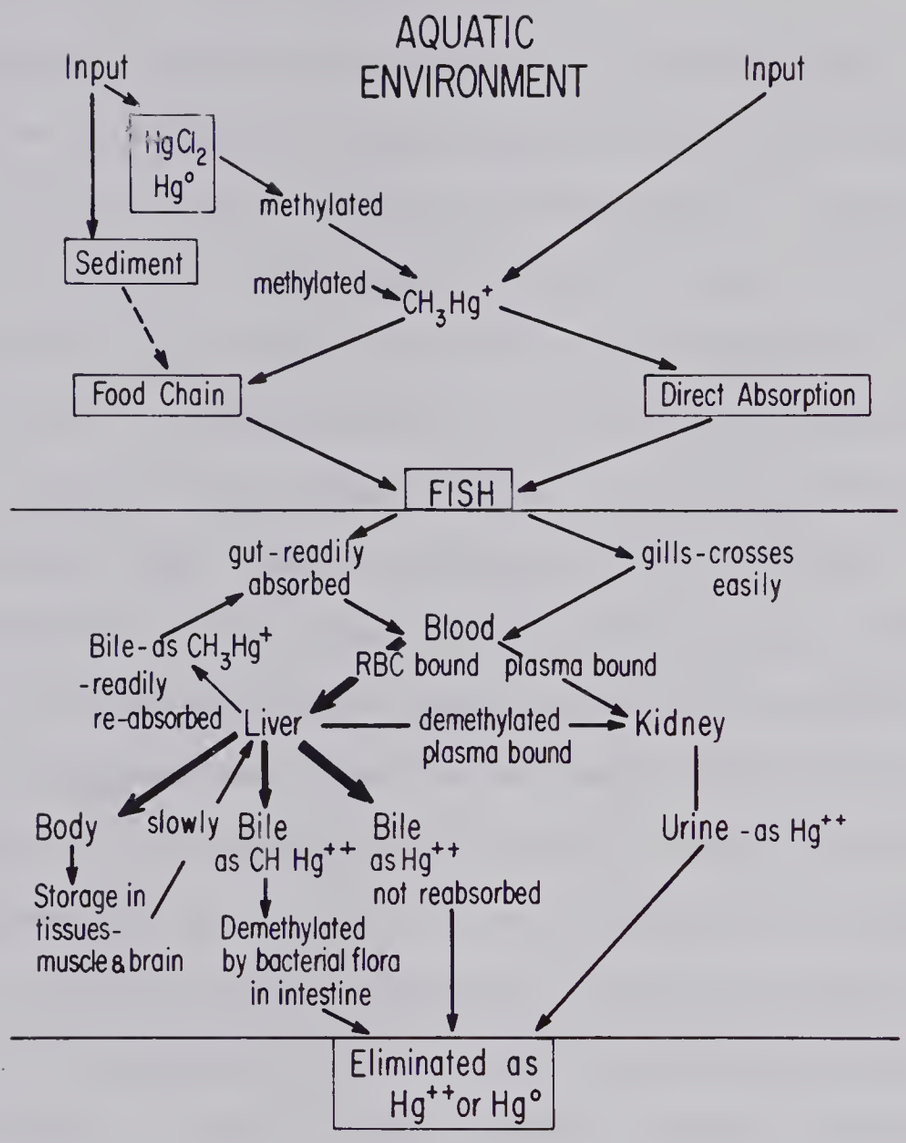


Figure 1 Mercury Uptake Distribution and Elimination Routes in Fish.

that mercury which is present in the fish, is primarily in the methylated form (Lockhart et al., 1972; Jernelöv, 1972).

Once present in the body, most probably in the methylated form, mercury will be transported to other tissues via the blood, where it is bound to the proteins in the cellular fraction (Giblin & Massaro, 1973). Binding to the cellular fraction rather than the plasma, the mercury will be less susceptible to elimination via the kidneys in the urine. Inorganic mercury, when present, is carried primarily by the plasma fraction and is therefore readily excreted by the kidney (Giblin & Massaro, 1973; Goodman & Gilman, 1975). Backstrom (1969), Norseth & Clarkson (1970), Burrows & Krenkel (1973), Dickinson & Krenkel (1973), McKim et al., (1976) have all suggested, on occasion that methyl mercury is, in all probability, demethylated in the liver and subsequently bound to the plasma fraction of the blood and thereby eliminated by the kidney in the urine. It could also be passed into the gastrointestinal tract in the bile, where being in the inorganic form, the mercury would not be readily reabsorbed by the gut and would therefore be eliminated in the feces. That is to say there would be little enterohepatic circulation. Giblin & Massaro (1973) states that the main excretion route of methylmercury in rainbow trout is through the feces. Dickinson and Krenkel (1973) also states that fish do not readily reabsorb excreted mercury because it is in the inorganic form.

When dealing with relative tissue/organ levels, these authors suggest that the kidney, liver and blood would show the highest levels 2-4 days after acute exposure to mercurial compounds. This would then gradually decrease with time (60-90 da). If, however, the exposure is continued chronically, these tissues would maintain a steady state level, albeit elevated, but there would occur a significant linear increase in the levels of mercury found in skeletal muscle and brain. Once located in these tissues,

the mercury has a slow turnover rate and half-lives have been estimated at up to 800 day (Lockhart et al., 1972; Jernelöv, 1972; Giblin & Massaro, 1973).

Short term (< 7 da) experiments, employing a single acute dose of mercury, resulted in the highest levels being found in the blood, liver, kidney and spleen coupled with accompanying low levels in the skeletal muscle and brain (Weisbart, 1973). But Giblin & Massaro (1973) state that after 100 da chronic exposure, the skeletal muscle, which comprises 55% of the body weight of rainbow trout contained 50% of the mercury.

It should be remembered that the hypothesis presented here has been drawn from many diverse sources, dealing with animals, from quail to blue gills, encompassing homeotherms and poikilotherms. It is entirely possible that the actual events are not this simple. However, as with most preliminary investigations where model building may not solve the problem, it does provide an organized means of testing the different parameters involved.

In 1973 the mercury levels found in goldeye sampled from the North Saskatchewan River were at levels which caused a considerable degree of concern (Munson & Daniel, 1973). The origin of the mercury found in the goldeye was of particular interest and was narrowed down to two probable sources. Besides the possibility of goldeye migrating into Alberta already possessing high levels of mercury, there may exist some peculiar aspect of goldeye physiology which permits high tissue levels to occur from the very low levels of mercury present in Alberta (Munson & Daniel, 1973).

The former hypothesis has been discussed in considerable detail elsewhere (Chapter III) and this particular chapter will deal with the physiology of goldeye as it pertains to mercury metabolism and excretion.

The optimal experimental situation would involve chronic studies conducted over a period in excess of 100 da. However, due to limitations of

time and space only acute studies were conducted.

In an attempt to determine whether goldeye indeed possessed some peculiar means for retention of mercury, two species of fish, goldeye and rainbow trout, *Salmo gairdneri*, were acutely exposed to a single dose of $^{203}Hg\ (NO_3)_2$ or $CH_3\ ^{203}Hg\ Cl$ and subsequently held for up to ten days. Mercury-203 elimination in the urine was monitored and tissue distribution of the isotope was determined upon completion of each experiment.

The remainder of this chapter will be concerned with the results of these experiments and a comparison of the data with these which have been published.

MATERIALS AND METHODS

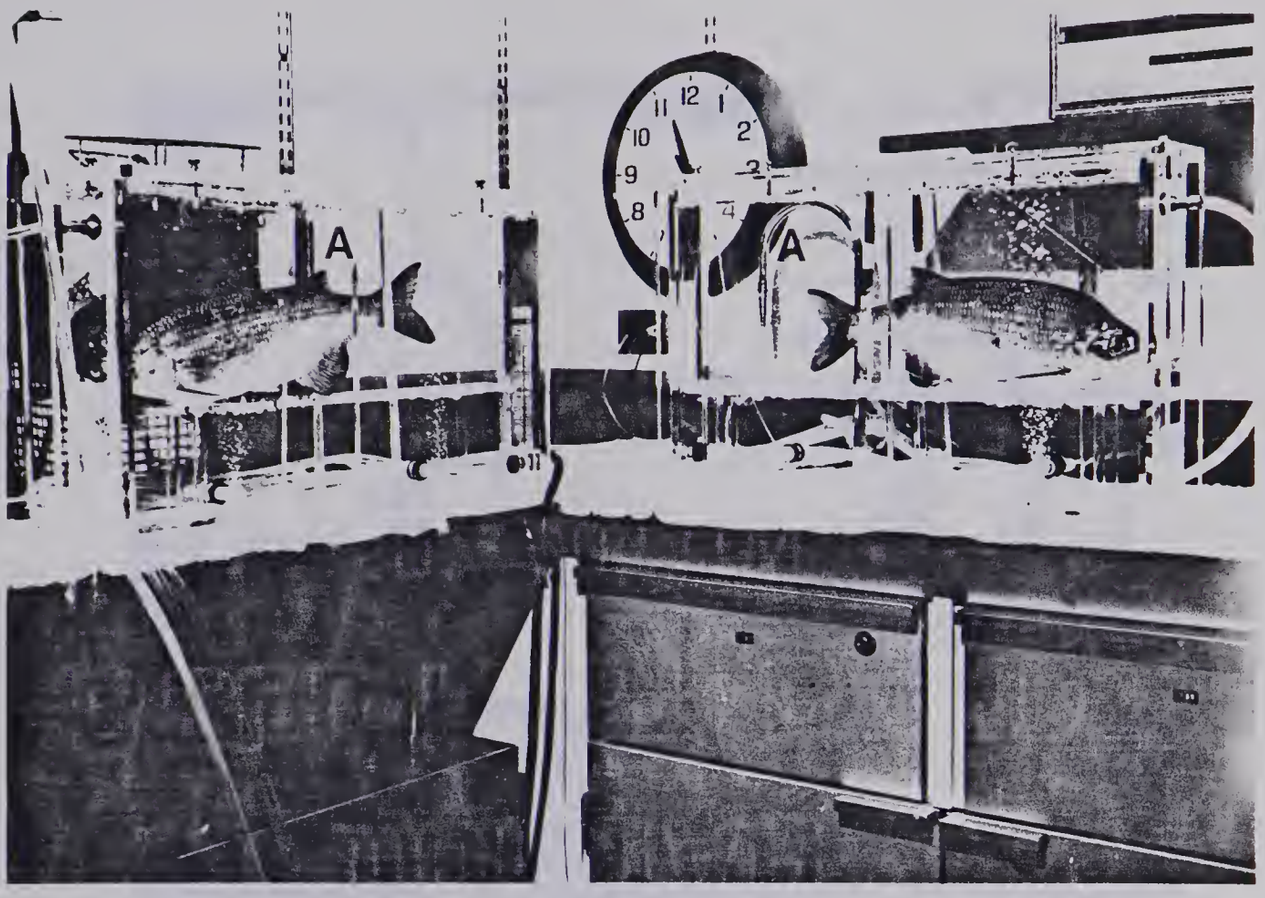
Goldeye, *Hiodon alosoides*, were captured from the North Sasktchewan River upstream of Edmonton, utilizing 300 m of continuous gill net of 2.5 cm stretched mesh, 1.8-2.4 m in depth. The fish were removed from the net within 5 min of capture, placed in a five gallon covered container and immediately conveyed to a portable 100 gal covered tank which served as a transportation tank when moving the fish from the field to the laboratory. All goldeye utilized were in excess of 4 years of age. The goldeye were acclimated at 5°C in 200 gal continuous flow holding tanks for at least 1 week prior to entering the experiment.

Rainbow trout, *Salmo gairdneri* were obtained from a hatchery as yearlings and were of uniform weight when used, approximately 300 gm.

The water in the holding tanks originated from the city of Edmonton water supply and was subsequently dechlorinated by passage through activated charcoal filters. The pH was adjusted by acid drip using muriatic acid and the pH and the chlorine content were monitored 24 hrs/da. The water was chilled to 5°C by passage through external cooling coils.

A urethral catheter (goldeye-PE60 intermedic tubing, trout PE90) was inserted into the urinary bladder and sutured in place using 000 silk sutures. The above procedure was conducted under tricaine methanesulfonate (MS222) anesthesia at 20,000:1 concentration. The fish were then placed in sealed plexiglass experimental tanks, measuring 50 x 8 x 30 cm with 10 l capacity (plates 1-3). The tanks were aerated with double sintered polyethylene stones which were placed in the base of the tanks. The water was flowing continually through an external glass wool filter and external cooling coils which maintained the tank temperature at 10°C. The coils were cooled by a Hetofrig-Heto Birkeröd Denmark, No. 1174 cooling pump.

Plate 1



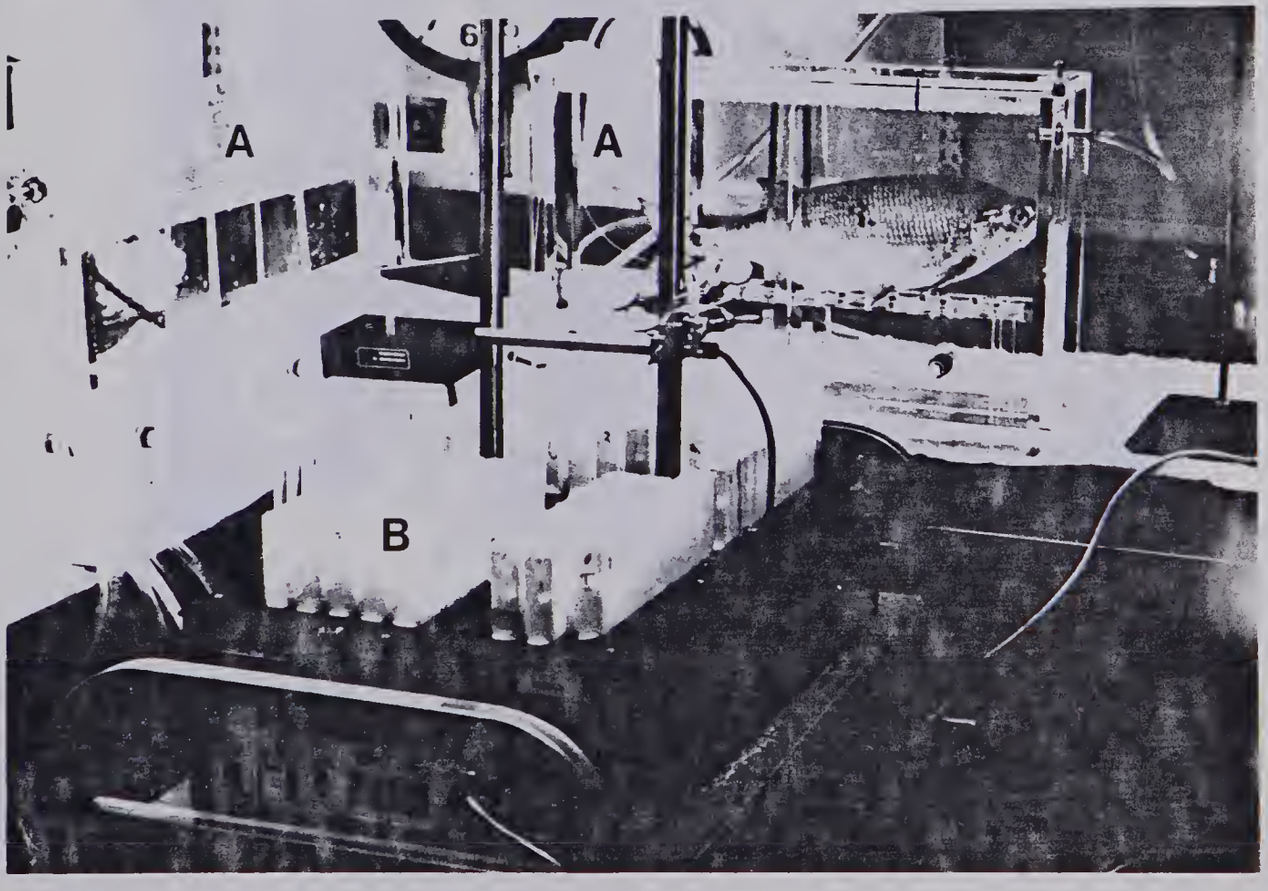


Plate 2

Plate 3

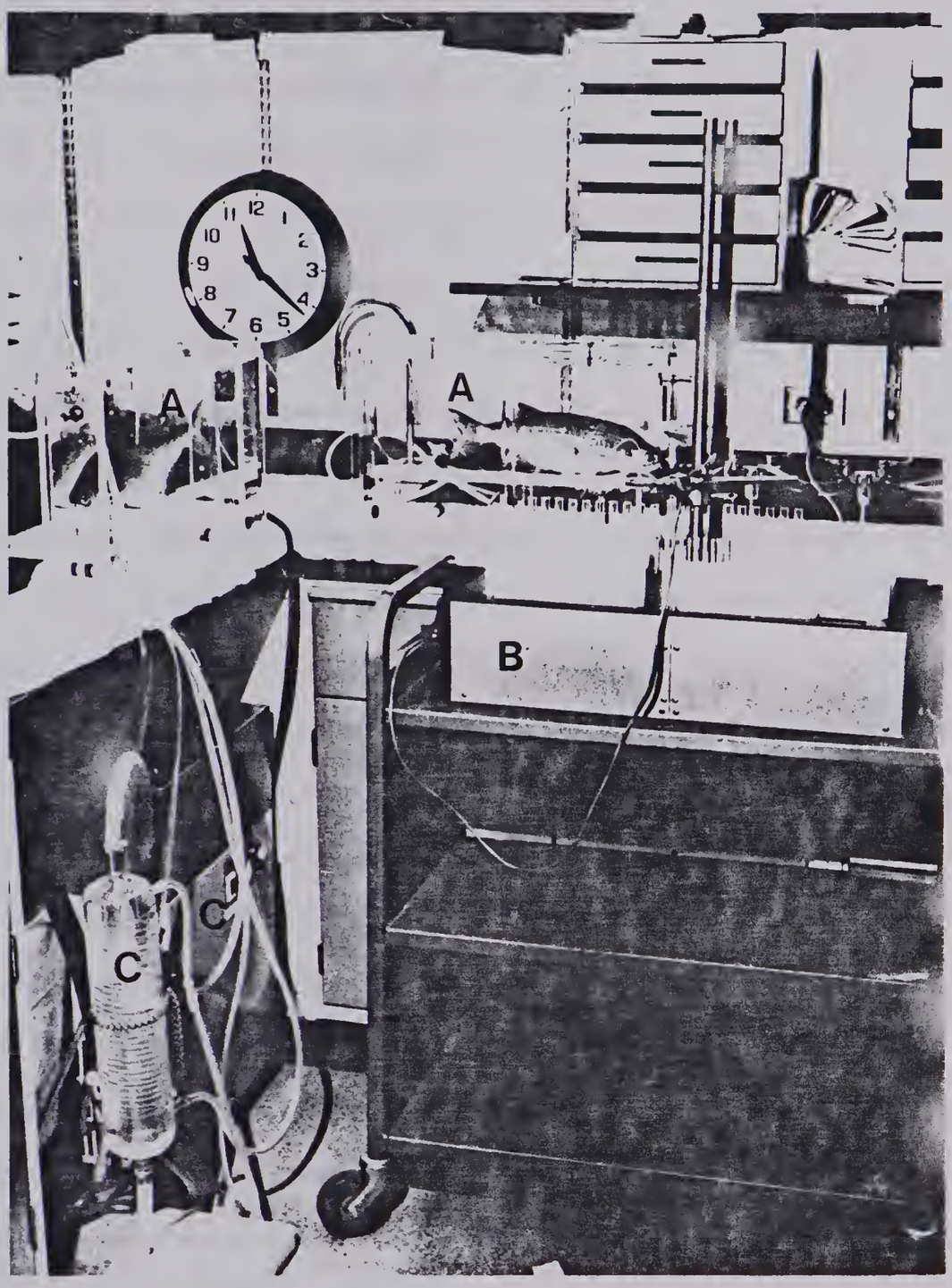


Plate 1 - A - Experimental Tanks

Plate 2 - B - Urine Fraction Collector

Plate 3 - A - Experimental Tanks
- B - Urine Fraction Collector
- C - Cooling Pump and Coil

The water in these tanks was treated similarly to that in the holding tanks and both the water and the glass wool filters were changed daily. 24 hrs was allowed for acclimation to 10°C and the urine collected during this time was utilized for control and isotope counting efficiency determinations. Following the initial 24 hrs the fish were again anesthetized with MS 222 and an intraperitoneal injection of methylmercuric-203 chloride (1.0×10^9 dpm) or mercuric-203 nitrate (1.0×10^9 dpm) was administered. The isotopes were supplied by ICN Pharmaceuticals Inc., Irvine, California, with specific activities of 3.05 Ci/gm Hg for CH_3 Hg Cl and 5.69 Ci/g Hg for Hg $(NO3)_2$. All radioactive counting was conducted using a Picker spectro scaler III-deep well gamma ray spectrophotometer utilizing a NaI crystal. Counting efficiency of this system was 2-3% as determined by external and internal standards using both urine and tissue samples.

Urine was collected hourly, 24 hr/da by a Golden Retriever Fraction Collector, Model 327, Instrumentation for Scientific Research. The radioactivity of the samples was counted regularly 3-4 times each day.

Upon termination of the experiment, the fish, again anesthetized with MS 222, was weighed and a blood sample was taken by caudal vein puncture using a 5 cc syring and a #21 gauge needle. The fish was then killed by severing the head and duplicate tissue samples were taken. The tissues were weighed and the amount of radioactivity was determined with the gamma ray spectrophotometer.

RESULTS

The rate of organic and inorganic mercury elimination in goldeye is illustrated in Figure 2 and Table 1. The ordinate is expressed in % dose as determined by the following formula:

$$\frac{\text{DPM/hr in urine}}{\text{DPM injected}} \times 100$$

Figure 3 illustrates the rate of inorgainic mercury eliminated via the urine in trout.

Experimental difficulties, coupled with a shortage of methylmercuric-203 chloride, were experienced in the studies using trout. In consequence, no data regarding the rate of methylmercury excretion via the urine were determined for this species.

In goldeye, mercury, resulting from the injection of both the inorganic and organic forms, began to appear in the urine within the first hour following injection. Within 5 hours mercury from the inorganic injection showed a 10-fold increase in the urine when compared to that for organic mercury (Fig. 2). Mercury from the inorganic injection rapidly reached peak values in the urine within the initial five hours post injection. This, however, descended sharply to a plateau which was maintained for the duration of the experiment. Mercury in the urine following injection of organic mercury never reached a peak value but appeared to rise slowly over the initial 10 hours to a plateau which was then maintained for the duration of the experiment.

Trout appeared to eliminate mercury resulting from inorganic injection in a manner similar to goldeye, but in larger quantities, by a factor of ten (Fig. 3, Table 1). It should be noted that only 0.08% of the inorganic dose and 0.02% of the organic dose were eliminated in the urine by goldeye over the test period. Trout on the other hand, eliminated 2.0% of the inorganic dose in the urine over the test period.

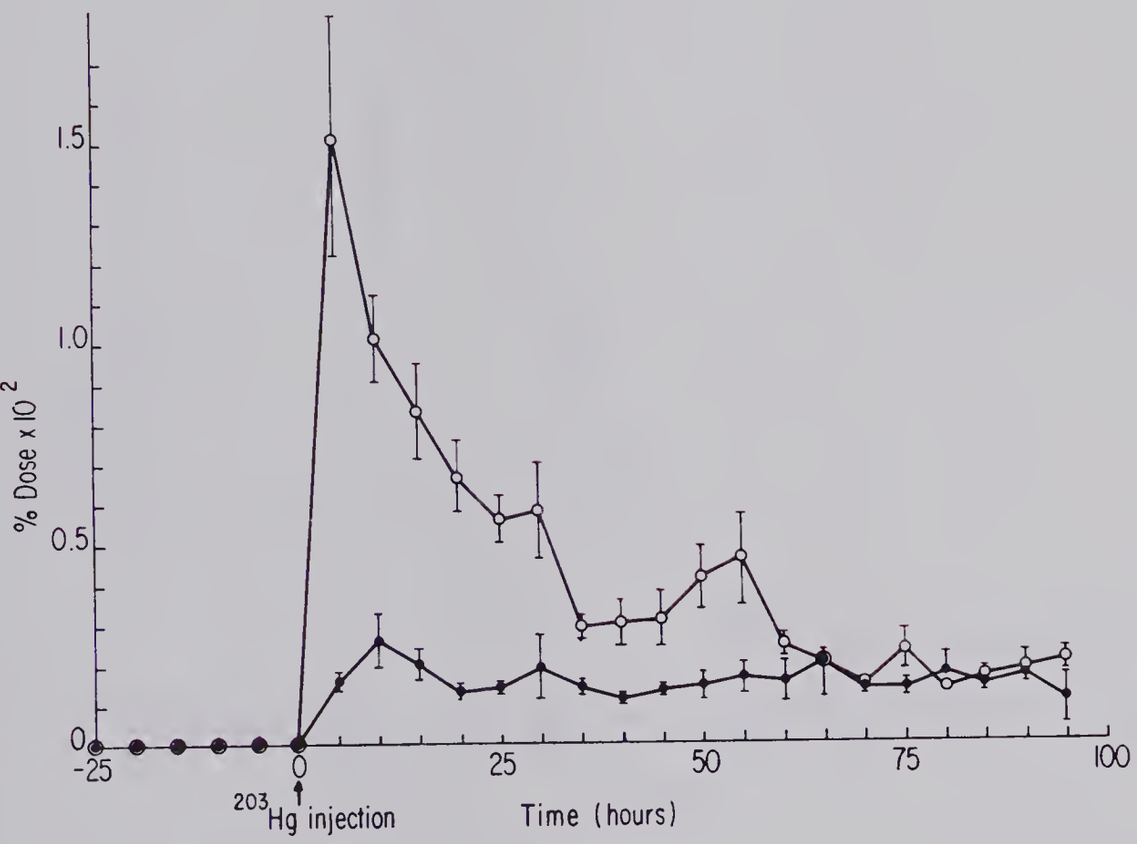


Figure 2 Mercury-203, Inorganic and Organic, Levels in the Urine of Goldeye Following Intraperitoneal Injection of the Radiolabel (% dose $\pm$ SE)

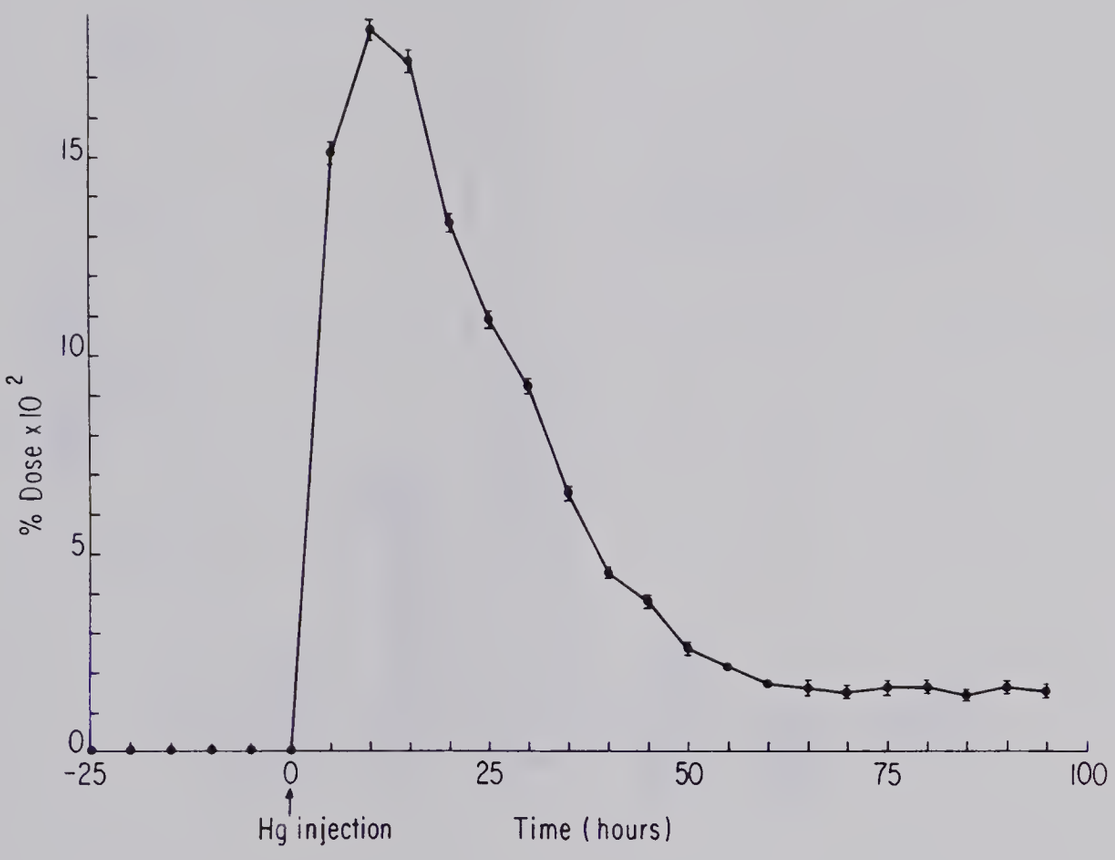


Figure 3 Inorganic mercury-203 in the Urine of Rainbow Trout Following Intraperitoneal Injection of the Radiolabel. (% dose $\pm$ SE)

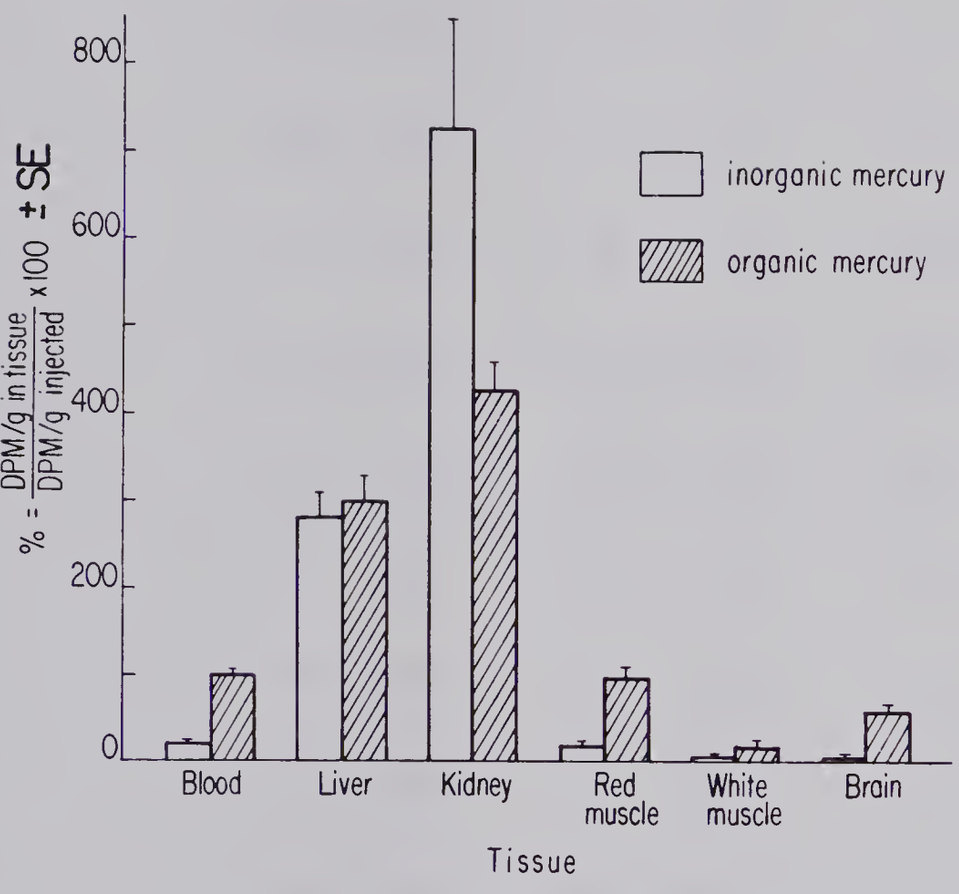


Figure 4 Distribution of mercury-203, Inorganic and Organic, in the Tissues of Goldeye 100 hrs after Intraperitoneal Injection of the Radiolabel

TABLE 1

Rate of mercury, organic and inorganic, elimination in the urine of goldeye and trout
% dose x 10^2 ± S.E.

	Goldeye		Trout
Hrs after Inj	CH_3 ^{203}Hg	^{203}Hg	^{203}Hg
5	0.16 ± 0.03	1.51 ± 0.28	15.14 ± 2.50
10	0.26 ± 0.07	1.05 ± 0.12	19.70 ± 2.70
15	0.20 ± 0.04	0.83 ± 0.12	18.34 ± 2.79
20	0.13 ± 0.02	0.67 ± 0.09	13.33 ± 2.06
25	0.14 ± 0.02	0.56 ± 0.06	10.92 ± 1.90
30	0.18 ± 0.08	0.58 ± 0.12	9.16 ± 1.36
35	0.14 ± 0.02	0.29 ± 0.03	6.48 ± 0.99
40	0.11 ± 0.01	0.30 ± 0.06	4.48 ± 0.74
45	0.13 ± 0.01	0.31 ± 0.07	3.79 ± 0.54
50	0.14 ± 0.04	0.41 ± 0.08	2.56 ± 0.48
55	0.16 ± 0.04	0.46 ± 0.11	2.14 ± 0.33
60	0.15 ± 0.03	0.24 ± 0.03	1.65 ± 0.24
65	0.20 ± 0.09	0.20 ± 0.03	1.61 ± 0.27
70	0.13 ± 0.01	0.14 ± 0.01	1.46 ± 0.15
75	0.13 ± 0.02	0.23 ± 0.05	1.63 ± 0.17
80	0.17 ± 0.05	0.13 ± 0.01	1.58 ± 0.20

Tissues

The results for mercury content of tissues were expressed as a percentage of the weight specific injected dose. This percentage was calculated in the following manner.

$$\frac{\text{DPM/g in tissue}}{\text{DPM/g injected}} \times 100$$

A value of 100% assumes equal distribution throughout the body and a value of greater than 100% infers selective accumulation in that particular tissue. A value of less than 100% in any particular tissue suggests less than equal distribution.

Mercury levels in the kidney, liver, following injection of either organic or inorganic radio-labelled mercury, were indicative of selective accumulation (Fig. 4, Table 2 & 3). This situation was common to both goldeye and trout.

Mercury concentrations in the blood of goldeye were not indicative of selective accumulation whereas blood levels of mercury in trout were indicative of selective accumulation. However the levels of mercury in the blood of both species of fish were significantly higher following organic mercury injection than they were following the injection of inorganic mercury.

Mercury concentrations in the kidney were significantly higher following the injection of inorganic mercury where compared to the levels following the injection of organic mercury. This was consistent both for trout and goldeye (Table 2 & 3) ($P < 0.01$).

Mercury levels following organic mercury injection, were significantly higher in the red and white muscle and brain when compared to levels in similar tissues following injection of inorganic mercury. This relationship was common both to goldeye and trout (Table 2 & 3) ($P < 0.01$).

TABLE 2

Tissue distribution of ^{203}Hg 100 hr following intraperitoneal injection of labelled organic (methyl) and inorganic mercury in goldeye, % dose x 10^{2} ± S.E.

Tissue	Inorganic	Organic
Blood	28.1 ± 5.3	100.0 ± 8.6
Bile	---	115.2 ± 29.9
Liver	277.9 ± 33.3	299.6 ± 33.1
Kidney	729.4 ± 166.7	426.0 ± 33.3
Red muscle	11.9 ± 2.2	96.1 ± 11.6
White muscle	4.4 ± 1.3	11.7 ± 3.0
Brain	5.12 ± 1.8	57.1 ± 69
Lens	0.00	0.00

TABLE 3

Tissue distribution of ^{203}Hg 100 hr following intraperitoneal injection of labelled organic (methyl) and inorganic mercury in trout, - % dose x 10^{2} ± S.E.

Tissue	Inorganic n=8	Organic n = 2
Blood	110.1 ± 19.5	307.0 ± 39.5
Bile	54.8 ± 20.7	45.0 ± 0.89
Liver	404.3 ± 81.3	509.0 ± 77.0
Kidney	1345.3 ± 328.4	535.5 ± 27.5
Spleen	1069.0 ± 404.0	428.1 ± 152.2
Red muscle	26.4 ± 8.5	303.5 ± 97.5
White muscle	5.9 ± 1.9	12.5 ± 2.5
Brain	15.9 ± 2.3	82.0 ± 7.99
Lens	0.00	0.00

Therefore the relative tissue distributions of both inorganic and organic mercury appeared very similar in both species of fish, trout and goldeye. Trout, however, appeared to contain a greater percentage of mercury in all tissues examined when compared to goldeye.

It is interesting to note that in both cases the lens of the eye never possessed levels significantly above background.

In both goldeye and trout, the distribution of mercury in blood fractions was similar, with mercury resulting from inorganic injection being distributed 50% in plasma and 50% in the cellular fraction. Mercury resulting from organic injection was distributed 10% in the plasma and 90% in the cellular fraction.

Both goldeye and trout contained significant levels of mercury in the bile but the actual contribution of the bile to total elimination proved impossible to determine as the functioning of the gall bladder, in terms of emptying times, is at present unknown for the species of fish tested.

In goldeye, 40% of the total organic mercury injected was recovered while 50% of the total inorganic mercury injected was recovered. In trout, 72% of the total inorganic mercury injected was recovered and no recovery values were determined for organic mercury because of the low number tested (2) and lack of urine samples.

DISCUSSION

This work appears to be the first to directly determine mercury elimination via the urine in fish. By various indirect methods several authors (Giblin & Massaro, 1973; Weisbart, 1973; McKim et al., 1976) have suggested that the amount of mercury actively eliminated in the urine would probably be minimal. They also suggest that the rate of elimination would show an initial peak followed by a subsequent fall to a plateau which would be maintained for a considerable period of time. They predicted that inorganic mercury would show a more rapid removal in the urine than organic mercury. Data obtained for both trout and goldeye (Fig. 2 & 3, Table 1) appear to agree with the above statements.

The theory being suggested is that mercury, which is eliminated in the urine, is in the inorganic form. The basis for this statement involves combining several different observations and ideas. Following inorganic mercury injection, mercury levels in the urine attained higher levels more quickly and the kidney contained significantly higher levels as compared to those for organic mercury injection. Inorganic mercury, being primarily bound a plasma component (Goodman & Gilman, 1975) would be more susceptible to elimination by the kidney in the urine, whereas organic mercury, bound to the cellular fraction of the blood, would not. It has also been suggested that demethylation occurs in the liver and subsequent transport via the plasma would result in increased levels in the kidney coupled with an increased rate of elimination in the urine.

At least two previous authors (Giblin & Massaro, 1973) have stated that the majority of mercury eliminated from the body occurs via the feces. This implies the existence of a connection between the circulatory system and the gastrointestinal tract whereby mercury could be eliminated. The obvious choice is the bile. Giblin and Massaro (1973) and McKim et al. (1976) report high

mercury levels in the bile and the present study also found high mercury levels in the bile of both species of fish tested. The chemical form of mercury in the bile is, at present, unknown, however if the bile was to function as an elimination route, the primary form must be inorganic. This is based on the fact that inorganic mercury is poorly reabsorbed from the gut, whereas, if the mercury existed solely or in part in the methylated form, then a high percentage of the mercury would be reabsorbed from the gut and therefore not eliminated.

There does exist another possibility which would enable the bile to function as an excretory route for mercury and which would not be dependent on the chemical form of the mercury in the bile. The bacterial flora present in the gastrointestinal tract could demethylate organic mercury present in the gut contents thereby reducing any subsequent reabsorbtion and enhancing mercury elimination in the feces.

Another suspected route of elimination would be via the gill system. McKim *et al.*, (1976) have stated that the gills certainly appear to function as a source of entry, especially to methlymercury. McKim states that after exposure ceased, the mercury levels in the gills dropped to levels no higher than those found in the blood. The gills were not examined in the present study primarily because they are richly vascularized and would therefore contian high levels of mercury because of perfusion of mercury contaiminated blood.

The relative ease with which methylmercury crossess cellular membranes and the blood-brain barrier would lead one to predict that skeletal muscle, red and white, and the brain would receive higher levels of mercury when exposed to methylmercury as opposed to inorganic mercury. Both species of fish examined in this study support this concept.

The liver, being richly perfused by the blood, and a suspected site of demethylation, would be expected to contain elevated levels and show very little difference between which chemical form of mercury was introduced. The data for both goldeye and trout show the liver to possess levels indicative of selective accumulation but possessing very little preference as to which chemical form the mercury was in.

The kidney, as would be expected, contained high levels of mercury, in both species tested. According to the concepts outlined above, mercury in the inorganic form, associated with plasma components, would be subjected to filtration and subsequent elimination in the urine. Therefore inorganic mercury would be expected to show higher levels in the kidney than organic mercury. Data obtained for goldeye and trout in the present study confirm this supposition.

Red muscle, which is tonic in function and richly vascularized, would be expected to contain higher levels of mercury than white muscle regardless of which chemical form the mercury is in. In both species of fish tested, red muscle contained significantly more mercury than white muscle.

However it was noted that the levels of mercury in red and white muscle of both species of fish were significantly increased when the injected mercury was methylated as opposed to inorganic mercury. This observation tends to support the concept that methylmercury crosses cellular membranes much more readily than inorganic mercury in similar systems.

It has been stated frequently throughout this text that, possible and probable demethylation of methyl mercurials, occurs in the liver. It is, however, just as conceivable to suggest that inorganic mercurials could be subject to methylation. This could account for observed gradual increase in mercury levels in various tissues following inorganic mercury injections even though inorganic mercurials do not readily cross cellular membranes.

In all cases the lens of the eye showed no significant uptaken of mercury, regardless of chemical form. This is a surprising finding because Giblin & Massaro (1973) have reported high levels in the eyes of experimental fishes and it was also determined that the lens of the eye of goldeye from the natural environment contained significant levels of mercury (Chapter III).

The comparison of the data for trout and goldeye showed differences, in quantitative terms only, for a few tissues, which in all probability were due to the size of the trout utilized (avg wt 200 g) as compared to goldeye (avg wt 550 g) when both species received similar dosages. Most significant, however, is the fact that the distrbution of both chemical forms of mercury in the tissues of both species of fish was identical.

The original problem was concerned with whether goldeye exhibited some peculiar aspect, in terms of their physiology, which would explain the presence of high levels of mercury in their tissues, from very low level environment contamination (Munson and Daniel, 1973). The results of these experiments suggest that goldeye exhibit tissue distribution and elimination routes for mercury which are strikingly similar to that reproted in the literature for other species and to that exhibited by rainbow trout, which was exposed to similar forms of mercury under identical conditions.

On the basis of these studies we have no evidence to suggest that goldeye show significant differences in the distribution or excretion of mercury when compared to other species, and thus it would appear that such an explanation cannot account for the high levels of mercury observed in goldeye from the natural environment. It msut be remembered, however, that thie conclusion is based on acute parenteral exposure and it is possible, if unlikely, that a different situation pertains during chronic exposure.

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CHAPTER V

FINAL SUMMARY AND DISCUSSION

INTRODUCTION

The results of the survey conducted in 1973 by Munson & Daniel revealed a series of unusual findings regarding mercury contamination of the North Saskatchewan River in Alberta. These authors showed that bottom sediments, invertebrates and river water from the North Saskatchewan River possessed mercury levels similar to background, indicative of low level contamination.

Goldeye contained mercury levels significantly higher than any other species of fish. This fact appeared inconsistent with the rapidly accumulating body of information regarding the distribution of mercury through a fish population in a contaminated area. The concept that the specific trophic level location of any given species of fish would determine the degree to which that species would be contaminated did not appear to be supported by the data collected from the fish fauna in the North Saskatchewan River. Northern pike and walleye are located above goldeye on the food chain but contained significantly less mercury than goldeye. In fact most of the other fish species contained mercury levels more indicative of background levels in a non-contaminated environment.

In view of this data several questions became evident. If the North Saskatchewan River in Alberta is as free from mercury contamination as it appears to be, where then does the mercury in goldeye originate? As the mercury levels in goldeye do not appear to follow food chain magnification, is there some physiological characteristic peculiar to goldeye which would permit uptake and retention of mercury from an environment exhibiting only background levels of mercury contamination? Is the food chain magnification theory valid?

Data from contaminated areas where goldeye have been investigated have revealed that goldeye appear to follow the typical food chain magnification concepts regarding mercury contamination (Bligh, 1970; Wobester et al., 1970; Sumner et al., 1970). If this is the case, then what unique characteristics exist, either in the North Saskatchewan River in Alberta or in the biology and behavior of goldeye which would explain the observed data?

It was therefore, the aim of this thesis to attempt to answer some of the above questions and thereby determine the source of the mercury contamination which had given rise to the elevated mercury levels in goldeye from the North Saskatchewan River in Alberta.

In order to supply adequate answers to the above questions, two hypotheses have been formulated and subsequent investigations were aimed at confirming, rejecting or modifying these hypotheses. First, goldye may have been exposed to mercury contamination prior to migrating to Alberta or second, goldeye may, through some peculiar or specific physiological function, accumulate and retain mercury from the background levels existing in the North Saskatchewan River in Alberta.

First Hypothesis

In order for the first hypothesis to be valid three criteria must be fulfilled: the goldeye population or significant portions of it, must not permanently reside in Alberta but be a migratory population which freely moves within the Saskatchewan River system in Alberta and Saskatchewan. Second, there must exist a significant source of mercury contamination which is within migratable distances of Alberta and third, mercury must possess an extended half-life in the skeletal muscle of these fish.

These three criteria as they apply to goldeye in the North Saskatchewan River will be dealt with in considerable detail.

Migratory Population of Goldeye

The question of goldeye migratory behavioral patterns in the Saskatchewan River system reveals the definite lack of adequate data concerning the biology of goldeye in this particular river system.

The initial investigations on goldeye in western Canada were conducted by Battle and Sprules in 1947 and 1948. This work was updated and rewritten by Kennedy and Sprules in 1967. These authors laid the ground work by describing morphological and meristic characteristics and noting some aspects of behavior.

Paterson (1966) published a note concerning goldeye in the North Saskatchewan River where he states that the youngest goldeye sampled in Alberta was in the 3-4 year age class. He also stated that goldeye do not appear to successfully spawn in this particular river. Kooyman (1972) and Donald (1972) working on goldeye from the Peace-Athabasca Delta, noted that goldeye appear to undertake lengthy river migrations to spawn in the spring. Fernet (1973), working in the Delta and also in the North and South Saskatchewan Rivers, also notes goldeye migrations involving considerable distances. Kraft (pers comm) tagging goldeye in the Medicine River and Red Deer River also documented lengthy migrations. All of these investigators suggest goldeye undertake yearly upstream migrations associated with annual spring spawning, followed by a subsequent downstream migration in the fall to suitable overwintering areas. In the Peace-Athabasca Delta this appears to be the Peace River itself while in the Saskatchewan River system either Tobin Lake or Diefenbaker Lake (a reservoir created by the Gardner Dam on the South Saskatchewan River) appear to be the most logical choices.

In order to support the concept that the goldeye population in Alberta may be a dynamic one rather than solely a resident population

a tagging and recapture study on the natural population of goldeye was conducted and described earlier in this thesis.

A model has been synthesized in an attempt to explain the composition of the goldeye population in Alberta. This model utilizes the available facts, coupled with a generous serving of conjecture, as a preliminary attempt at explaining the available field data. Further investigation may require that alterations, in whole or in part, be made to this model. It does, however, provide a basis from which subsequent investigative planning can be made.

Goldeye Population Model

The goldeye present in Alberta are a spawning population and enter this province in large numbers, from an overwintereing area, such as a lake or reservoir, for the sole purpose of spawning. Spawning commences within a couple of weeks following breakup of the river ice in the spring. Spawning encompasses a period of six weeks which corresponds with the time peak flow rate of the North Saskatchewan River.

No single cue for spawning has been identified; however, it probably encompasses a combination of several physical events. These events probably include photoperiod, water temperature, water velocity and turbidity. It is obvious that an inter-relationship exists between each of these parameters and the others.

Upon expulsion and fertilization, the egg, or zygote, being single and semi-buoyant, drifts with the current. Hatching occurs within 27-29 days of fertilization (Kennedy & Sprules, 1967), by which time the eggs would be well into Saskatchewan. The fry would continue to be carried by the current until they reached Tobin Lake, which would provide an ideal location for subsequent growth and overwintering.

Young-of-the-year goldeye would be expected to be found in Tobin Lake in August but should be completely absent from the upstream spawning areas.

The young-of-the-year goldeye would then spend the following two years in Tobin Lake and with approaching sexual maturity, which occurs at 3-4 years (Kennedy & Sprules, 1967), these goldeye would commence an upstream migration. The velocity of the river may require that this migration encompass two seasons.

This migration would then place the young goldeye in Alberta at age 4 which was the youngest goldeye caught in Alberta and is also the age at which sexual maturity occurs.

From the data concerning gonad development, it is evident that goldeye spawn every year but whether adult fish undergo such vast annual migrations is at present unknown.

Spawning behavior of goldeye has not been observed however the following SPECULATION is offered as one possible description of spawning events.

Spawning probably occurs in backwater areas where the current is at minimum. Each ripe female possess aproximately 20,000 eggs which are expelled singly or in pairs. As the eggs are expelled from the females they are fertilized by the male which has assumed a side-by-side position. There is probably some inter-digitation of the anal fins, accounting for the sexual dimorphism exhibited by this appendage. Floating, single eggs would prove impossible to fertilize once free from the female but the side-by-side posture would solve the proximity problem as well as being much more efficient, especially in view of the small volume of sperm produced by the male. Also because of the vast numbers of eggs produced by each female it is entirely possible that several males would fertilize the eggs produced by one female (certainly a genetic advantage). This would suggest that a breeding population should contain many more males than females or that males possess the ability to spawn more than once.

This interpretation of the data suggests that there exists a single dynamic population of goldeye in the Saskatchewan River system. The population of goldeye in Alberta is, therefore, dependent upon recruitment of migrating goldeye from Saskatchewan, even though spawning does occur in Alberta. This model is not completely substantiated by direct evidence; it is however the most convenient one embodying the observed data. There are, however, elements of the dynamic population concept which proved to be testable.

1. The population of goldeye in Alberta was in continual movement as evidenced by tag returns. Even in the instances where tags were returned from the same general area that the fish were released, 3 weeks to 2 months had elapsed between release and capture.

2. Tagging data conclusively proved that individual goldeye are capable of migrating considerable distances up to 2000 km in 15 days.

3. Tag return data from Alberta further suggests that an upstream movement occurred during spawning time (May and June) followed by a downstream movement in subsequent months (table 3, Chapter II). These data were further enhanced by the tremendous increase in the frequency distribution observed during the spawning period and was followed by a substantial decline in catches in the later months (figure 8, Chapter II). If Edmonton area is the upper limits of goldeye migration, that is the spawning area, then one would expect to observe massive influx of goldeye during the spawning periods followed by general decline in numbers during the subsequent months.

4. If goldeye move up the North Saskatchewan River to spawn each year then sexually mature males and females in spawning conditions should be found in abundance at spawning time in Alberta. This observation was consistent for each year that sampling was conducted. If goldeye do indeed move upstream to spawn then there should exist a significant

difference between fish populations in Alberta and Saskatchewan at spawning time. In May and June size distribution and age of goldeye at Nipawin was different from the population in the Edmonton area (figure 7, 8 & 9 Chapter II). Also, if a downstream movement to a suitable wintering area occurred in late summer then the population of goldeye sampled in August and September at Nipawin should be significantly different from the early spring population. Analysis of the size distribution or age of goldeye at Nipawin revealed that there did exist a significant difference between the early spring and late summer populations (figures 9 & 10, Chapter II). However, examination of these figures suggests that several interpretations are possible where the most probable one can only be identified by further data accumulation at this particular site.

5. No direct evidence was obtained that the spawning of goldeye, which does occur in Alberta, was successful. This was primarily due to the existence of the single, semibuoyant, floating egg of goldeye which rendered evaluation of spawning success very difficult to determine. However, the success of a fertilized free floating egg would be manifested by the presence of young-of-the-year a considerable distance downstream from the spawning area and their virtual exclusion in the spawning areas. The North Saskatchewan River is a fast flowing turbulent river where free floating eggs could be carried considerable distances and young fry would not attempt any vast upstream movement. The logical result of these circumstances would be the presence of young-of-the-year in Saskatchewan (figure 10, Chapter II) and their exclusion from Alberta (Paterson, 1966; figures 6-8, Chapter II).

Source of Mercury

Tobin Lake, a reservoir created by the Squaw Rapids Dam on the Saskatchewan River, has a history of significant mercury contamination in bottom sediments and fish fauna (Sumner et al., 1970; Wobester et al., 1970; Bligh, 1970; Atton & Fernet, pers. comm.). These workers have determined that the chlor-alkali plant in Saskatoon, which discharges its effluent into the South Saskatchewan River, is the source.

Mercury Half-Life

There is ample evidence for the extended half-life of mercury in the skeletal muscle of fish, where half-lives have been estimated to exceed six hundred days (Hannerez, 1968; Jernelöv, 1972; Lockhart et al., 1972). However, no investigations have been conducted on the half-life of mercury in the skeletal muscle of goldeye.

Thus, this and other work has shown that the three criteria required for the validity of the first hypothesis have been satisfied. Therefore the concept that the high mercury levels found in goldeye in 1973 in Alberta has originated from contamination arising from outside the provincial borders, is, at least, reasonable.

Mercury Contamination

The levels of mercury contamination in a dynamic population of goldeye would lead one to predict that insignificant differences would exist in mercury levels in goldeye from Edmonton and Nipawin, while stationary species should show a steady decline in mercury levels as the distance from the sources increases.

Goldeye sampled from Edmonton and Nipawin in 1974 and 1975 showed no significant differences in mercury levels (Table 1, Chapater III) while sauger and walleye sampled from Nipawin in 1974 possess significantly higher mercury levels than similar species sampled in Edmonton in 1974 (Table 2 & 3, Chapter III).

There was a significant decrease in mercury levels of all species of fish sampled at Nipawin (with the possible exception of suckers) from 1974 to 1975 presumably as a result of reduced environmental contamination which will be discussed later. The decrease in mercury levels in goldeye from Nipawin was paralleled by a similar decrease in mercury levels in goldeye from Alberta (table 1, Chapter III). However, the decrease in the mercury levels in sauger and walleye from Nipawin was not paralleled by a similar decrease in mercury levels in these fish in Alberta (table 2 & 3, Chapter III). These data again support the concept that the goldeye population to the two provinces is indeed a dynamic one.

The observed drop in the mercury levels in virtually all species of fish sampled in Saskatchewan appears to be related, at least in time, to the cessation of mercury output from the chlor-alkali plant in Saskatoon in 1972-1973. There appears to be a time lag of approximately one year before a significant decrease in the mercury content of skeletal muscle of fish was observed.

The more interesting note here was the rapidity of the recovery of the fish fauna. Armstrong et al. (1972) states what many investigators believe to be true, that is, the rate of recovery of bottom sediments following massive chronic mercury input, will take decades to return to normal background levels. Also it is recognized that bacteria in the bottom muds possess the ability to methylate inorganic mercury (the most frequent chemical form in industrial effluents), thereby converting the mercury to a form which is much more mobile in an aquatic environment (Wood et al., 1968). These facts are not disputed in this thesis but their relative importance in terms of absolute levels of mercury contributed to the water and the food chain is certainly questioned. It seems quite possible that the

quantitative contribution of bacteria-mediated methylation of mercury to the overall levels of mercury in fish, may, in fact, be minimal. The most important aspect of mercury contamination is, in all probability, the actual levels in the ambient water itself. The rate of methylation and mobilization of mercury into the ambient water and food chain from the bottom sediments known to be bacterially mediated (Wood et al., 1968) and therefore subject to temperature changes may be relatively unimportant to the overall contribution of mercury, especially in a cold environment.

Although the food chain is a recognized pathway for mercury movement in most environments, its relative importance in the aquatic environment is similarly questioned. There exists no doubt that the food chain does contribute to the levels of mercury in the aquatic fauna, especially those at the top, however the levels of mercury in the ambient water are, in all probability, the factor which will contribute most significantly to the levels of mercury found in fish (Giblin & Massaro, 1973; McKim et al., 1976).

Tissue Distribution

High levels of mercury in certain internal organs and tissues are indicative of more recent exposure. This may be due to a rapid turnover rate because these tissues function as centers of excretion, e.g., liver and kidney. It may also be due to the particular tissue developing over a relatively short period of time, e.g., gonads. In goldeye the female gonad develops to virtual maturity from July to September. Therefore mercury levels in this particular tissue would be indicative of exposure to mercury over a relative short period of time.

Brain, skeletal muscle and lens of the eye possess very slow turnover rates and therefore levels of mercury in these particular tissues would be indicative of exposure over an extended period of time.

This study revealed that goldeye from both Alberta and Saskatchewan possessed high levels of mercury in the skeletal muscle and lens of the eye (table 6, Chapter III). These results suggest either exposure to significant levels of mercury had occurred over an extended period of time or exposure to very high levels of mercury had occurred at one particular stage in their life history.

However, when tissues possessing a more rapid turnover of mercury, such as kidney and liver, were examined in goldeye from the two provinces significant differences were noted (table 6, Chapter III). Mercury levels in the kidney, liver and gonad of goldeye from Alberta in 1974 were very low, even though high levels of mercury were found in the white skeletal muscle and the lens of the eye. Mercury levels in the kidney, liver and gonad from goldeye from Saskatchewan, were significantly higher than the levels in similar tissues in goldeye from Alberta, while the levels in the lens and muscle not significantly different (table 6, Chapter III).

These results further support the concept that the environment from which goldeye were sampled in Alberta was relatively mercury free, while the situation in Saskatchewan, at least in 1974, still retained an indication of contamination.

These results indicate that the tissue to be examined for mercury levels must be chosen with care. Analysis for mercury levels in skeletal muscle, especially white, and lens of the eye will provide an indication only of the degree of exposure which had occurred in the preceding two to four years. The mercury levels in slow turnover tissues, such as these, are not adequate indicators of the degree of mercury contamination in the particular environment from which they were taken, especially if the fish species being examined has a history of undertaking lengthy annual migrations.

Analysis for mercury in tissues where mercury has a short half-life such as the kidney, liver and gonad provide a much more valid indicator of the current mercury levels in the particular environment from which the fish were taken.

Therefore it becomes highly significant to determine not only the dynamics of mercury distribution in the specific tissues being examined, but also adequate biological and behavioral data on the species being examined must also be obtained.

While these studies have not established beyond any doubt that the mercury, in the 1973 goldeye sampled from Alberta, had its origin in Saskatchewan this remains an explanation which is consistent with all the data so far obtained.

SECOND HYPOTHESIS

The second hypothesis suggests that the high levels of mercury found in goldeye from Alberta in 1973 were due to retention and elimination rates peculiar and specific to goldeye. This hypothesis was directly testable in the laboratory. If goldeye do possess detectable differences in the metabolism and excretion of mercurial compounds then one would predict, that when compared to other species of fish, significant differences in tissue distribution and elimination rates would become evident. However, experiments utilizing radio-labelled ^{203}Hg, inorganic and organic conducted on both goldeye and rainbow trout revealed that the tissue distribution and rate of elimination of both chemical forms of mercury was similar for both species of fish (figure 2, 3, 4, table 2, 3, Chapter IV).

Although this project appears to be the first to attempt to directly determine the rate of mercury elimination in the urine of fish, the results substantiate the contribution to the overall excretion of mercury as predicted by recent authors (Giblin & Massaro, 1973; McKim *et al.*, 1976).

Tissue distribution and elimination in the urine of mercury by goldeye was similar to that of trout and agreed with the data published to date.

It was therefore determined that, based on these acute studies, goldeye do not appear to possess detectable unique or peculiar physiological capabilities in regards to the retention; distribution and elimination of mercury.

Therefore the combination of the biological and behavioral characteristics of goldeye in the North Saskatchewan River, coupled with tissue distribution of mercury in this species of fish, strongly suggests that the mercury in the goldeye sample of 1973 had its origin in Saskatchewan as the direct result of the severe contamination of the river system which had occurred prior to this date. The unique feature attributed to goldeye does not appear to be the rate of mercury metabolism and elimination, but rather the result of very specific and characteristic biological and behavioral traits.

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APPENDICES

FURTHER RESEARCH

Biology of Goldeye

To more completely delineate the status of goldeye in the Saskatchewan River System a much more indepth approach must be made at Nipawin-Tobin Lake in order to determine the following:

(a) composition of the goldeye population from May through November.

(b) special attention should be focused on the condition of the females during May and June, whether or not they do spawn in this area.

(c) determine the furthest upstream from Nipawin that young-of-the-year appear.

(d) conduct a much more intense effort into determining the behavior of goldeye during spawning.

(e) intensify efforts at locating free floating goldeye eggs via floating plankton nets and pusue artificial hatching experiments.

Mercury Analysis

With the observed decrease in mercury levels in the fish, which can only be attributed to the substantial curtailment of input of mercury at Saskatoon, further monitoring of the mercury levels in all species of fish in the Saskatchewan River System will provide valuable information in regards to the rate of recovery from mercury contamination of the river and fish fauna in it.

Laboratory Experiments

The following recommendations regarding further laboratory experiments are suggested, however it must be noted that in several cases the cost of conducting the experiments most probably outstrips the projected value in terms of information gained.

(a) conduct long-term (> 90 da) experiments with both acute and chronic exposure to the two most important mercury compounds, inorganic and organic.

(b) determine an accurate time course for the rate of mercury elimination in the urine from both acute and chronic exposure.

(c) determine the most significant route of elimination of mercury compounds, feces, urine or gill system.

(d) determine the most common form of mercury which is eliminated; that is, is it organic or inorganic.

(e) determine the route which would contribute to the highest levels in the body; direct exposure from the ambient environment or from indirect exposure via the food chain.

(f) do mercury compounds within the body affect behavior and perception and if so at what levels and to what extent?

(e) At what level, in terms of cell function, does the toxicity of mercury exert itself?

AIDS FOR FURTHER RESEARCH

Biology of Goldeye

Contrary to the available information, goldeye can be caught at anytime of the day by either gill net or line. A few hints for increasing the catch success are included.

(a) goldeye most probably do not learn about a gill net from a preceeding exposure, however it was determined that if they could see a net they would avoid it. That is catches increased at night and when Secchii disc readings exceeded 100 cm. In both cases visibility was decreased. With Secchii disc readings in excess of 100 cm success with the line was considerably decreased. At Secchii disc readings of 100 cm or less net catches decreased drastically while line successes increased. It is therefore suggested to make considerable use of the Secchii disc before deciding upon the most suitable method for capture.

(b) never set a gill net perpendicular to the current in the North Saskatchewan River and always secure one end to the bank.

(c) greatest net successes were obtained when the net was set approximately 2-5 meters from the current interface in a backwater area.

(d) the best method for capturing goldeye live for laboratory experiments with the minimum of damage is outlined below:

- use of a 1-2 cm stretched mesh net is highly recommended (line catching works but the numbers of fish obtained is minimal for the time invested). The fish must be cleared from the net almost immediately, transported in a small portable tank (20 l) to a large tank (> 400 l) for transportation to the laboratory. Wild fish should be held at 5-10°C for at least 1 week prior to use. Specimens were kept for up to 8 months without feeding. Feeding is highly recommended; however no completely successful means of accomplishing this in goldeye were found.

(e) at ambient temperatures above 15°C net mortality was extremely high and susceptability to fungus infection was greatly enhanced.

(f) artificial hatching experiments were a consistent failure primarily due to a fungus infestation, especially if the eggs were held in the river.

Mercury Determinations

All specimens to be utilized for mercury analysis must be frozen in plastic bags as soon after capture as possible. Mercury analysis data must be backed up by adequate meristic information on each and every fish (weight, length and age being a bare minimum.).

When analyzing for mercury it is highly recommended that two separate areas be utlized; one for weighing and chemical preparation and one for the actual analysis. The basis for this recommendation is that continuous contamination of the analysis equipment results when both are conducted in the same area. Because the equipment is so sensitive it takes very little contamination to completely negate a whole series of results. Contamination of all chemicals frequently and consistently occurs.

CATCH RECORDS

Species	Location	Date	Method
Mooneye *H. terigisus*	Big Island	July 10 - 1975	2½ gill net
"	Devon	July 30 - 1976	line
Quillback *Carpiodes cyprinus*	Big Island	Aug 6 - 1975	2½ gill net

All of these specimens are presently located in the museum of the Department of Zoology, University of Alberta, Edmonton, Alberta.